WATER

WATER

WATER

WATER

WATER

WATER

WATER

WATER

WATER

WATER

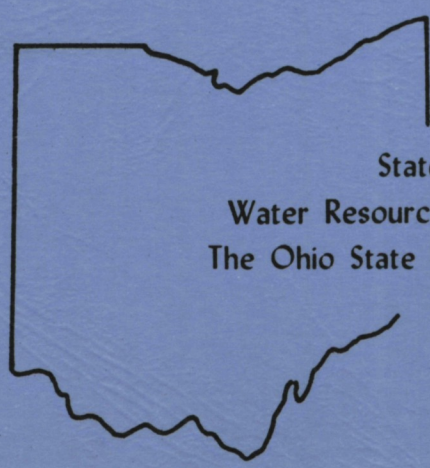
Project Completion
Report No. 710708

PREVENTION OF THE FORMATION OF ACID DRAINAGE FROM HIGH SULFUR COAL, COAL REFUSE AND COAL SPOILS BY INHIBITION OF IRON AND SULFUR OXIDIZING MICROORGANISMS

Patrick R. Dugan
Department of Microbiology
The Ohio State University
Columbus, Ohio 43210

United States
Department of the Interior

Contract No.
B-073-OHIO



State of Ohio
Water Resources Center
The Ohio State University

PREVENTION OF THE FORMATION OF ACID DRAINAGE FROM HIGH SULFUR COAL, COAL REFUSE AND COAL SPOILS BY INHIBITION OF IRON AND SULFUR OXIDIZING MICROORGANISMS

BY

PATRICK R. DUGAN

Department of Microbiology
And
Water Resources Center
The Ohio State University

FINAL PROJECT REPORT
PROJECT B-073-OHIO
Supported By The
OFFICE OF WATER RESOURCES RESEARCH
U.S. DEPARTMENT OF INTERIOR

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

DISCLAIMER

Contents of this publication do not necessarily reflect the views and policies of the U.S. Department of the Interior nor does mention of trade names of commercial products constitute their endorsement or recommendation for use by the U.S. Government.

ACKNOWLEDGEMENTS

Portions of the research included in this report were conducted by the following former students: Dr. William A. Apel, Mrs. Lynn Schirtzinger Apel, Dr. Alan DiSpirito, and Susan E. Dugan. The cooperation of the Peabody Coal Co. during early stages of this project is acknowledged.

The research on which this report is based was financed in part by the U.S. Department of the Interior, as authorized by the Water Research and Development Act of 1978 (P.L. 95-467).

INTRODUCTION

It has been estimated that 4 million tons of acidity drains into about 10,500 miles of streams in the Appalachian region each year. This polution spans 10,000 square miles across 11 states and also adversely affects 29,000 surface acres of reservoirs and other water impoundments (2).

Acid drainage is a problem associated geographically and geologically with the mining industry and is due to production of sulfuric acid from sulfur containing minerals.

Pyritic minerals and shale are often found geologically in intimate contact with coal or sandwiched between seams of coal. Some of the shale, reject coal, pyrite and other non-marketable minerals are extracted with the coal and left on the surface when the coal is taken to market. These piles of minerals, called **refuse piles**, and sometimes referred to as **gob piles**, may extend to several hundred acres. Several kinds of pyritic minerals are found in nature but iron pyrite (FeS_2), also known as fool's gold, is the one most commonly encountered in association with coal. There is no practical way to segregate pyrite from other minerals and low grade coal in refuse originating from either deep or drift mines.

When a coal seam is sufficiently near the surface, a large shovel or drag line can be used to scoop away the overburden and expose the seam of coal. It is practical to remove up to about 100 to 110 feet depth of overburden. Once the overburden has been stripped away by large shovels or draglines, smaller shovels follow behind and scoop up the coal seam and load it into trucks, railcars, etc. Consequently, overburden which may contain pyrite is piled in wind rows on the surface. It is often possible to separate much of the pyrite from other minerals in overburden during a stripping operation and bury it deep in the **spoil bank**. The term **spoil** is used in conjunction with stripped

overburden, in contrast to the refuse or "gob" from subsurface mines or from coal preparation plants.

Acid formation represents a major environmental problem in the Appalachian region where vast amounts of high sulfur coal are mined. The problem results from exposure of the pyritic minerals to the combined effects of atmospheric oxygen, moisture and a group of acidophilic iron and sulfur oxidizing bacteria (10,28). These bacteria catalyze the oxidation of pyritic minerals, principally iron pyrite, and marcasite to ferric sulfate and sulfuric acid (8,9) according to the following reactions:

(I) $Fe^{++} \longrightarrow Fe^{+++} + \text{electron}$

(II) $2\,S^{-2} + 3O_2 + 2H_2O \longrightarrow 2(SO_4^{-2}) + 15 \text{ electrons} + 4H^+$

(III) Sum: $FeS_2 + 3O_2 + 2H_2O \longrightarrow 2H_2SO_4 + Fe^{+++}$

The oxidized iron (Fe^{+3}) formed, subsequently reacts with water to produce ferric hydroxide and more acid according to the following equation:

(IV) $Fe^{+++} + 3H_2O \longrightarrow Fe(OH)_3 + 3H^+$

Anyone who has seen drainage from mining regions where pyrite has been exposed will recognize the yellowish to reddish brown precipitate, called yellowboy, that forms on streambeds. This is the $Fe(OH)_3$ formed in equation (IV) and is equivalent to rusted or oxidized iron. Ferric hydroxide also reacts with sulfuric acid to form ferric hydroxy-sulfate complexes according to equation (V), hence the difference in color and composition of precipitates depending upon environmental conditions. $Fe(OH)_2^+$ may also be present in acid

solution.

$$Fe(OH)_3 + 2H^+ + SO_4 = Fe(OH)(SO_4) + 2H_2O \qquad (V)$$

Increased coal mining in the region is likely to increase the exposure of coal refuse with a high pyritic content to the conditions that produce acidic drainage. In addition, large amounts of acid are already being produced from abandoned strip mines and refuse piles.

Reclamation laws in many states presently require that spoils from active strip mines be replaced, that the land be returned to its original contour and then re-vegetated; usually by planting grasses, legumes and trees. This means that spoil materials having potential for acid production must be burried at the bottom and the remaining overburden including any top soil, would be placed over the acid spoil and graded to the top of the high wall. Problems costly to the mine operators often develop six months to two years later. The re-graded spoils may subside over a period of time leaving exposed high wall and pyrite. Also, some of the pyrite near the surface may be exposed to air and water because of erosion or because it was never buried during the reclamation procedure. "Hot spots" of acid covering several hundred square feet often develop after a year or two and kill the new vegetation on the slope. Since mining permits require payment of a bond and vegetation will not grow on the hot spots, the bond money is not returned to the mine operator until the problem spots are reclaimed. It is often very difficult and sometimes impossible to get the necessary equipment for neutralizing, discing, and re-planting to the hot spot location because of steep terrain. The original regrading was probably done by a drag line and very likely would not be in the

vacinity a year later.

Refuse piles have considerably more potential for acid production than do strip mine spoils because of the higher content of pyritic minerals. The magnitude of acid drainage from abandoned refuse piles is also historically greater because coal was deep mined or drift mined prior to development of stripping technology and many of the old mines along with their refuse piles have long been abandoned. With no one laying claim to abandoned mines, which literally honeycomb hundreds of square miles in Appalachia, any reclamation effort reverts to taxpayer expense.

Moisture can penetrate all levels of the porous refuse pile and moisture retention, as well as moisture content, depends upon the composition of the pile (e.g. the clay, coal, pyrite and sandstone content). Oxygen ordinarily does not penetrate into the pile depth beyond about 12 inches and is limited by a zone defined as the oxygen barrier which results from compaction of fine sediments (40). It is the propensity of refuse piles to produce sulfuric acid via the oxidation of iron pyrite (or other sulfur containing minerals) according to equation III which is the primary basis of our biological concern with strip mining. The sulfuric acid leaches or is flushed out of the pile at a rate determined by local precipitation and ground water flow (24,40). However, the rate and amount of acid production within the piles is determined by many factors such as (A) the amount of pyrite; (B) particle size of pyrite; (C) presence of microorganisms which oxidize the pyrite; (D) depth of oxygen penetration; (E) moisture content of the pile; and (F) temperature range of the pile, and presumably other factors which we presently do not understand.

Acid appears to be produced at a relatively consistent rate in a given refuse pile and it can be washed out in a surge during a rainfall resulting in a rapid slug-dose of acid entering a drainage or receiving stream. Continued

high water flow due to precipitation will subsequently tend to dilute the acid concentration of the drainage (15,32,41).

Tests with controlled sprinkling of refuse piles indicated that about 25% of the water applied was retained in the refuse pile while 75% flushed through almost immediately (24,40). Acid loads averaging 305 lbs per acre per day have been shown to be flushed out of refuse piles and slug loads of as high as 6,600 lbs/acre/day have been recorded. The drainage from such piles may contain as much as: 60,000 mg $SO_4^=$ per liter (6%), 15,000 mg total iron per liter (1.5%) and 60,000 mg net acidity per liter (40).

It has been estimated that over 370,000 acres of strip mined land will require some form of reclamation in Ohio alone. Of this total, 180,000 acres are inactive or abandoned acres that discharge over 1 million lbs. of acid per day into Ohio streams and will require a major reclamation effort. The acid in the streams is highly corrosive to bridges, dams and other structures as well as to plumbing. The toxicity and hardness of the water restricts its use for irrigational and livestock watering purposes as well as for recreational purposes. In general, water contaminated by acid mine drainage seriously retards virtually all beneficial water uses at tremendous economic loss (27).

Microorganisms

Relative to the role of microorganisms in acid drainage, a distinction between two categories of microbes is made on the basis of nutritional requirements. Both types have been discussed extensively in the literature.

Autotrophic microorganisms are those organisms which need only carbon dioxide, the oxidation of minerals as their source of energy,and a few trace minerals and/or vitamins as additional nutrients. This type of microbe, which includes the acidiphilic Thiobacillus group of bacteria can therefore grow in

a minimal nutritional environment since all of the minimal requirements are readily available in drainage.

Included amongst the known iron and sulfur oxidizing microorganisms are: the acidophilic thiobacilli: **Thiobacillus ferrooxidans** and **T. Thiooxidans**; the iron oxidizing bacterium: **Leptospirillum ferrooxidans**, the acidophilic thermophilic archaebacteria: **Sulfolobus acidocaldarius** and **S. brierlei** as well as some bacteria whose taxonomic status is presently uncertain. Of the acidophilic microbes known to oxidize pyritic minerals, **Thiobacillus ferrooxidans** is considered to be the primary organism responsible for production of acidic drainage from high sulfur coal, coal refuse and pyritic spoil banks. Those thiobacilli which require a highly acidic environment, utilize the energy released from the oxidation of both iron and sulfur (FeS_2) for chemoautotrophic growth i.e. for the reduction of carbon dioxide as the source of cellular carbon.

Heterotrophic microbes are those which depend upon the oxidation of reduced organic compounds for their energy in addition to their cellular carbon requirements. They also have nutritional requirements for trace amounts of minerals and/or vitamins similar to those of the autotrophic organisms. In general, specific differences among species of heterotrophic microbes are reflected in differences among types of organic compounds required by each species. This category of organism is somewhat more fastidious nutritionally than the autotrophic category, and nutritional requirements vary widely.

It should be pointed out that all organisms do not fall neatly into one category or the other. Many organisms are known which have the facility to adapt either to an autotrophic or heterotrophic mode of existence and are referred to as **facultative autotrophs** or **facultative heterotrophs.**

Acidophilic bacteria of the Thiobacillus group have long been recognized as being associated with pyrite oxidation and acid production in coal mine spoils and refuse and can be readily isolated from acid mine drainage water. (3,10,14,23,29,30,35,38,41). The bacteria grow optimally in the pH range of 2.0 to 3.5. Maintenance of an adequate supply of Fe^{+2} as an energy source in the absence of high concentrations of organic material requires an environmental pH less than 4.0 because of the rapid auto-oxidation of Fe^{+2} in the presence of O_2 above pH 4.0.

The pH optima of all enzymes purified from T. ferrooxidans are considerably higher (i.e. pH 5.0 (5,13,25,33,34)) than the environmental pH (i.e. 3.0), with the possible exception of the crude preparation of the cell envelope associated iron oxidase reported by Bodo and Lundgren (optimum 2.5 to 3.5;(6)). This suggests that the cell envelope of obligate acidophiles selectively excludes high concentrations of H^+ (the smallest ion) from entering the cell; a conclusion which has been supported by more direct assessment of the internal pH of T. ferrooxidans cells when the external pH is considerably more acid (12). Beck suggested that T. ferrooxidans has a passive H^+ barrier since resting cells do not respire and are capable of surviving long periods of storage under acidic conditions (3).

Although iron pyrite will be oxidized chemically in the absence of bacteria, ultimately to $Fe(OH)_3$ and H_2SO_4; the bacteria catalyze the reaction and increase the rate of oxidation up to one million times the chemical rate under acidic conditions (37). Further, it has been shown that the iron oxidizing bacteria are more active than the sulfur oxidizing bacteria with respect to rates of pyrite oxidation (28). This led to speculation that the primary role of bacteria in pyrite oxidation was the production of ferric ions as shown in equation I and that the ferric ions thus produced, oxidized more pyrite with concommitant regeneration of ferrous ions. The re-cycled iron

could again be oxidized by the bacteria and the cycle would continue (35,36,37).

Singer and Stumm (37) have concluded that under acidic conditions below pH 4.0, the rate of pyrite oxidation by ferric ion is considerably greater than the rate of ferrous ion oxidation in the absence of bacteria. Therefore, the bacteria must catalyze the oxidation of ferrous to ferric ion in order to supply the Fe^{+3} to oxidize the pyrite. They, therefore concluded that the bacterially catalyzed reaction controls the rate of pyrite oxidation under acidic conditions. The mechanism of elemental sulfur oxidation by T.thiooxidans would be somewhat different in that sulfur is essentially insoluble, therefore requiring direct contact of bacterium to substrate (44).

Much of our knowledge of the microbial oxidation of pyrite has been based upon laboratory investigations of the activity of isolated or enriched cultures of bacteria and relatively little data is available on investigations in the field. One of the most convincing field studies showing the activity of bacteria in refuse piles was carried out by Belly and Brock (4). These investigators showed a strong correlation between uptake of $^{14}CO_2$ and most probable numbers (MPN) of iron oxidizing bacteria but not with the acid tolerant heterotrophic microorganisms which were also present in the refuse. They reported maximal $^{14}CO_2$ uptake in coal refuse 2 to 3 years old with only slight incorporation in fresh material or material 40 years old. Maximal uptake was always found in samples taken from the surface above 8 to 10 cm depth, at temperatures between 20 ^{o} and 30 ^{o}C and at moisture content of between 23 and 35%, all of which agree with data previously published on the basis of laboratory investigations (40).

As refuse piles dry out somewhat during periods of low precipitation yellow crystals of ferric sulfate can be observed forming on both horizontal and verticle surfaces of refuse materials often at depths of 10 to 12 feet.

This indicates that the moisture remaining in the pile is saturated with ferric sulfate and represents a stored acid potential.

Although actual bacterial catalysis of pyrite occurs near the surface of refuse piles, the high content of Fe^{+3} dissolved in acid may leach through the spoil banks and catalyze pyrite oxidation deep within the pile. Oxidation would take place at a slower rate within the pile resulting in a drainage relatively high in Fe^{+2} which could then be re-oxidized by bacteria in drainage streams.

In a continuous flow situation where approximately 10^{8} cells per milliliter are continuously being removed, a finite amount of pyritic material is continuously being oxidized by the bacteria. A gross calculation based on experimental data indicates that 0.16 g-moles of iron oxidized will yield the number of cells found in a gallon of water. For example if the flow of water away from the source is 100 gal./min, then 64 moles of iron would have been oxidized per minute to yield the cells being lost. The efficiency of energy conversion has been reported to be 10 to 30%; therefore 64 x 3 = 192 g-moles of iron would be the minimum Fe^{2+} oxidized per minute in the above example; 192 moles of Fe is the amount found in about 50 lb of pyrite. These calculations are only intended as illustrations, and no accounting has been made for energy released from the sulfide in pyrite, which is about 10 times greater per mole than that from Fe^{2+} iron. Assuming that all ferrous and sulfide in pyrite were oxidized to ferric and sulfate, it would have required oxidation of 5 to 50 lb of pyrite per minute to yield the acidophilic autotrophs in a stream having a flow of 100 gal/min.

It is concluded that biological oxidation is significant in proportion to nonbiological oxidation, and efforts must be expanded to determine the most effective means of inhibiting the oxidations. One promising means is via specific antimicrobial chemicals, provided that the compounds can be placed in

proximity to the target organisms in the field and are relatively specific for the target.

Inhibition of Microbial Acid Formation

It is possible to inhibit metabolism of the autotrophic iron- and sulfur-oxidizing bacteria in the laboratory with the use of chemicals which are quite innocuous to most other living organisms, e.g., alpha-keto acids, carboxylic acids, and sulfated anionic detergents. Preventative methods which utilize antimicrobial agents should prove successful at specific locations. That is, success of prevention of this type pollution would depend upon ability to inhibit causative bacterial metabolism at the origin. Locating and inhibiting the microbial activity should not be a problem in the case of gob piles and in spoil banks but may be quite difficult in the case of abandoned deep mines.

Preliminary evidence suggests that chemical inhibitors might be practical with reference to cost, availability and lack of toxicity for organisms other than the iron and sulfur oxidizers (14,15,19,41,42). Dugan and Lundgren reported that the anionic surfactants; alkylbenzene sulfonate (ABS) and sodium lauryl sulfate (SLS), were very active inhibitors of T. ferrooxidans when cultivated in the laboratory (14). Dugan subsequently reported on the effectiveness of ABS and SLS (19,22). As shown in Figures 1 and 2, five ppm ABS (approximately 1.4×10^{-5} M) effectively inhibits iron oxidation by a T. ferrooxidans cell suspension containing 4×10^{7} cells/ml. Figure 1B shows similar data indicating that SLS is an effective inhibitor at 2 ppm (approximately 7×10^{-6} M). However, the effectiveness of ABS was dependent upon the number of bacteria in suspension as indicated in Fig. 2A and 2B where the number of cells in suspension was doubled (2x) and quadrupled (4x). Non-ionic detergents and some sulfonated organic compounds were much less toxic to growth than either ABS or

SLS. It was also shown previously that increase in Eh in this system was correlated with iron oxidation and growth of the organism (14).

Subsequent laboratory investigations demonstrated that several different low molecular weight organic acids inhibited iron and sulfur oxidation as well as growth of T. ferrooxidans. For example, acetic, fumaric, formic and oxalacetic acids completely inhibited iron and sulfur oxidation and the bacteria when present in 10^{-2} to 10^{-4} molar concentrations. Hexanoic, lactic, malic, oxalic, pyruvic and succinic acids were slightly less effective at 10^{-2} to 10^{-4} concentrations (42,43).

Other investigators have demonstrated the inhibitory effects of several organic acids on both T. ferrooxidans and T. thiooxidans (7).

One practical consideration which should be mentioned is that various types of sewage sludge contain high percentages of volatile solids which have a significant content of organic acids. Addition of sludge to spoil banks would therefore tend to be inhibitory to the iron oxidizing bacteria and of course would add an organic or humic content to the spoils. Caution must be exercised relative to the presence of viruses, pathogenic microorganisms and toxic minerals which may be present in certain sludges.

Figure 1

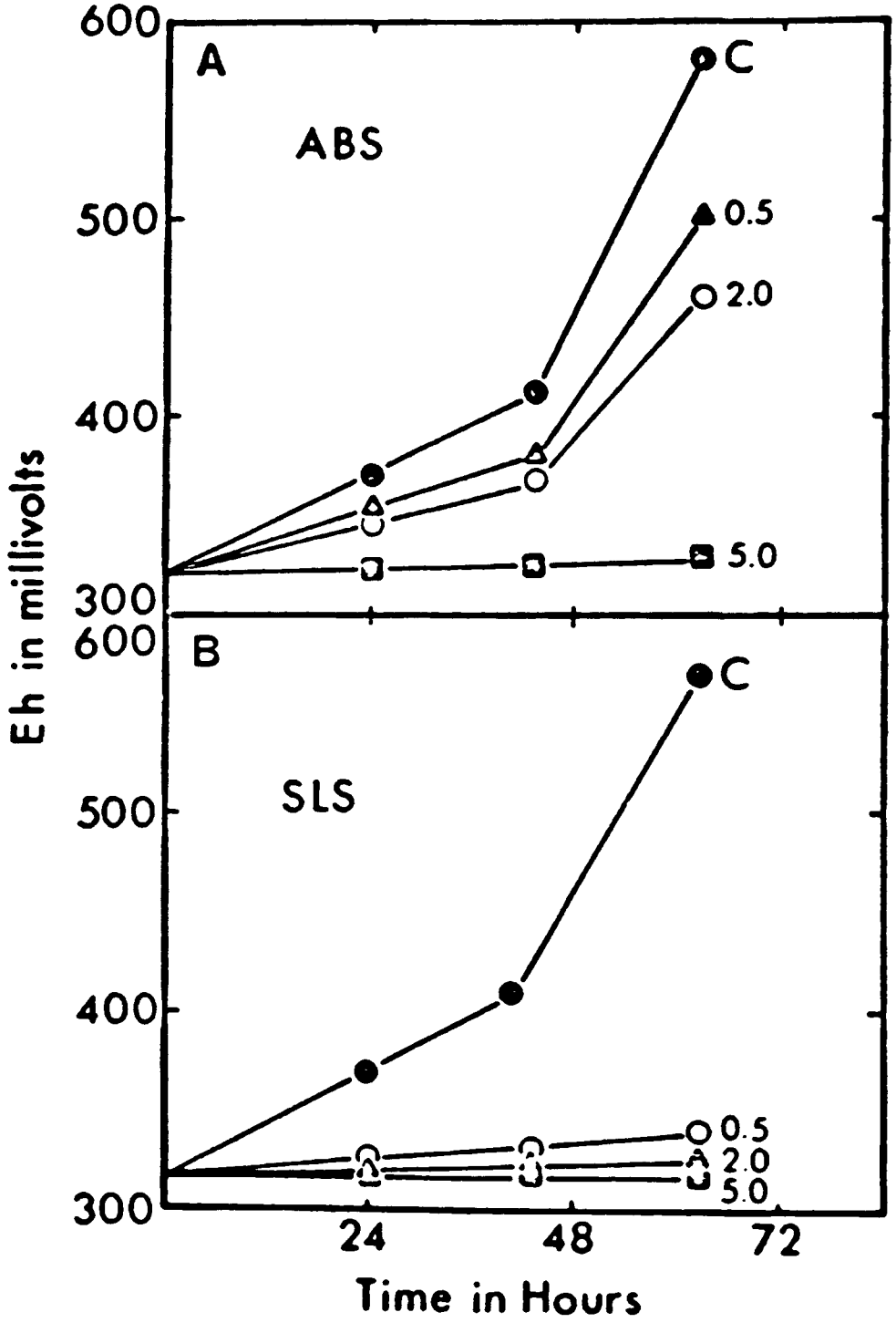
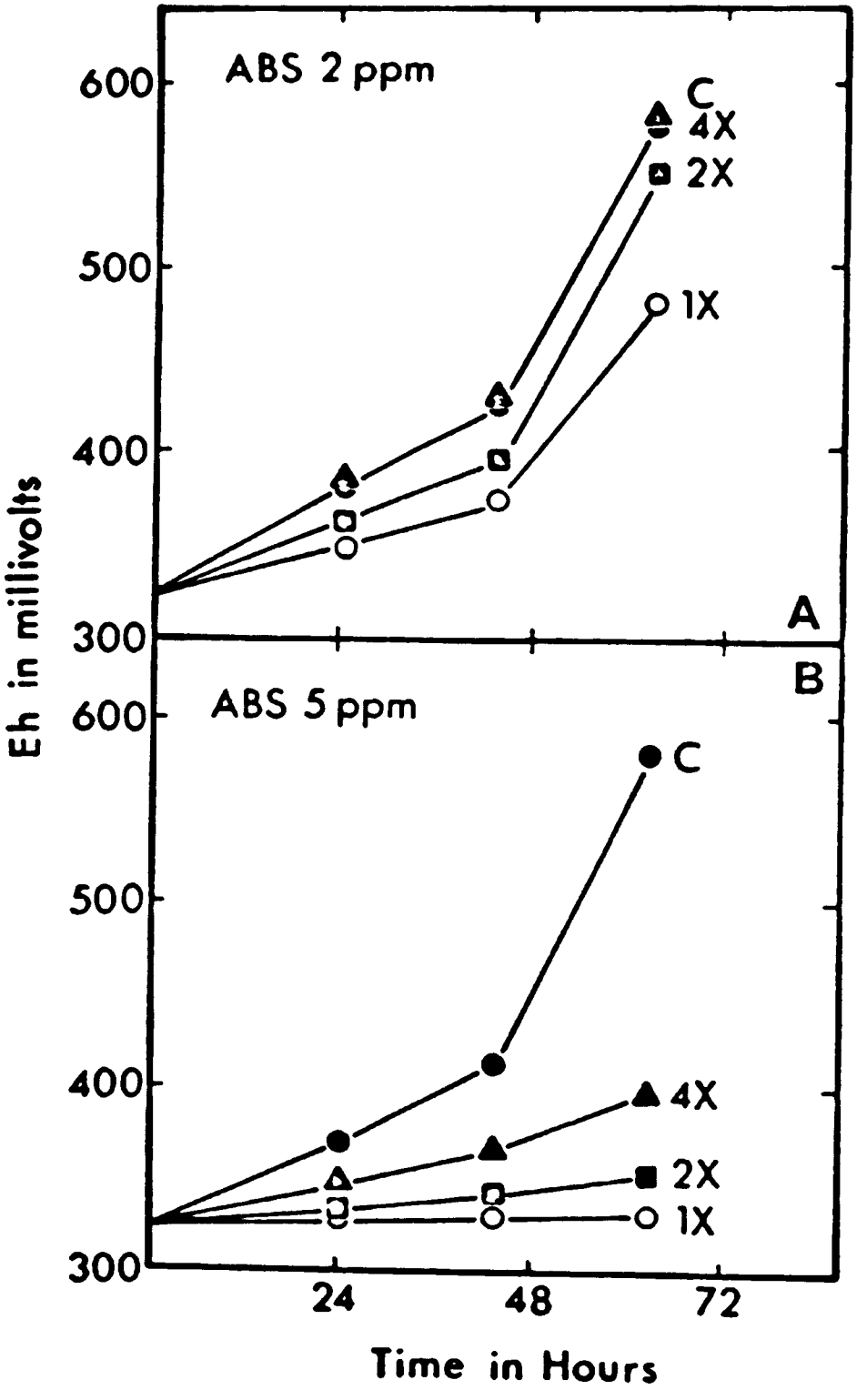

600
500
400
300
A
ABS
C
0.5
2.0
5.0
B
SLS
C
0.5
2.0
5.0
Eh in millivolts
24
48
72
Time in Hours

Figure 2


ABS 2 ppm
600
500
400
300
C
4X
2X
1X
A
B
ABS 5 ppm
600
500
400
300
C
4X
2X
1X
24
48
72
Eh in millivolts
Time in Hours

OBJECTIVE OF RESEARCH

The primary objective of this research was to demonstrate that sulfuric acid which forms as the result of microbial oxidation of pyritic minerals (e.g. acidic mine drainage) can be partially prevented by the application of specific microbial inhibitors.

It has been postulated that it may be possible to chemically inhibit the iron and sulfur oxidizing bacteria in the field and thereby reduce formation of acid drainage (19,22,42,43). Although inhibition of pyrite oxidation by inhibiting the acidophilic Thiobacillus species has been studied on a small scale in the laboratory, the potential for inhibition of the bacteria in their natural environments (e.g., coal refuse, abandoned strip mines, exposed underground coal seams, etc.) needs further examination. To be of utility in the natural ecosystem, chemical inhibitors must be relatively: (i) non-toxic to organisms other than the target microorganisms (ii) inexpensive, (iii) available in commercial quantity, (iv) amenable to application techniques, and (v) inhibitory to the acid producing bacteria in low concentration. Preliminary laboratory experiments indicated that the anionic detergents sodium lauryl sulfate (SLS), linear alkylbenzene sulfonate (LAS), and alkylbenzene sulfonate had considerable potential in this regard (19,22). However, these chemicals are biodegradable and heterotrophic bacteria, yeasts and filamentous fungi with potential for biodegradation of the detergents are also found in the highly acidic coal environments along with the Thiobacillus species (16,17). The heterotrophic organisms are known to indirectly stimulate pyrite oxidation by the iron and sulfur oxidizers by metabollically removing autotoxic metabolic by-products produced by the autotrophs and by providing trace nutrients to the autotrophs (21). Also, contact between chemical inhibitor and target organisms in the field is entirely different from highly controlled experiments in the laboratory.

EXPERIMENTAL

Previous research has established that **Thiobacillus ferrooxidans**, a chemoautotrophic iron and sulfur oxidizing bacterium, as well as related iron and/or sulfur oxidizers are key agents responsible for the oxidation of the pyritic minerals present in coal, coal refuse and other mineral refuse to ferric iron, sulfate and sulfuric acid. It has also been established that both the formation of sulfate and acid, determined by pH measurment, are adequate measurements to evaluate the course of pyrite oxidation because the only source of sulfate and acid in the experimental system is from the oxidation of pyritic mineral. Consequently, pH and $SO_4^=$ formation are utilized in this report to follow the oxidation of pyrite present in high sulfur coal refuse samples. This in turn provides for the estimation of pyrite oxidation rates in the presence and absence of iron and sulfur oxidizing microorganisms. It further provides for rapid analysis of the effects of chemical inhibitors on the overall metabolic activity of the iron and sulfur oxidizers during their oxidative attack on the pyrite present in coal and coal refuse.

EXPERIMENTAL PROCEDURES AND RESULTS

Control and Background Evaluations

Preliminary experiments were conducted on coal refuse obtained from the Peabody Coal Company, Sunnyhill Mine located near New Lexington, Ohio. Refuse was pulverized by grinding in the laboratory to pass a 150 mesh seive.

Effect of Washing. In order to evaluate the ability of microbial inhibitors to reduce formation of acid and concomitant production of sulfate salts in high sulfur coal refuse, it was necessary to wash out acid and soluble sulfate already present in the material.

750 gm. pulverized refuse was washed with 10 successive 3 liter volumes of distilled water. The pH and sulfate were measured on each of the 10 volumes of wash water. These data are shown in Figure 3 as pH and milligrams of sulfate per liter (x 1000).

Effect of Sterilization. The effect of sterilization by autoclave (20 lb. pressure for 20 min. at 250 ^{o}C) on the pH and sulfate found in coal refuse was recorded. Triplicate samples of 20% aqueous refuse suspensions (wt. to volume) were mixed at 600 rev. per min. for 20 hours in a Microferm fermenter and analysed with the following result:

Pre-sterilization, avg. pH 2.19, sulfate 4 gm/L.

Post-sterilization, avg. pH 2.51, sulfate 2 gm/L.

It was concluded that under autoclave conditions some of the acid present in the refuse was neutralized probably by reaction of sulfuric acid thereby decreasing the concentration of soluble sulfate.

The effect of autoclave sterilization on the pH and sulfate values of selected microbial inhibitor solutions was also evaluated and these values are shown in Table 1. Sterilization lowered the pH of the ABS and LAS solutions, possibly by reacting some of the alkaline salts. The sulfate concentration did not change as a result of autoclaving. In the case of lignin sulfonate formulations (Polyfon, Reax and Indulin) the pH did not change appreciably but free sulfate increased - suggesting hydrolysis of sulfate from the lignin.

Figure 3

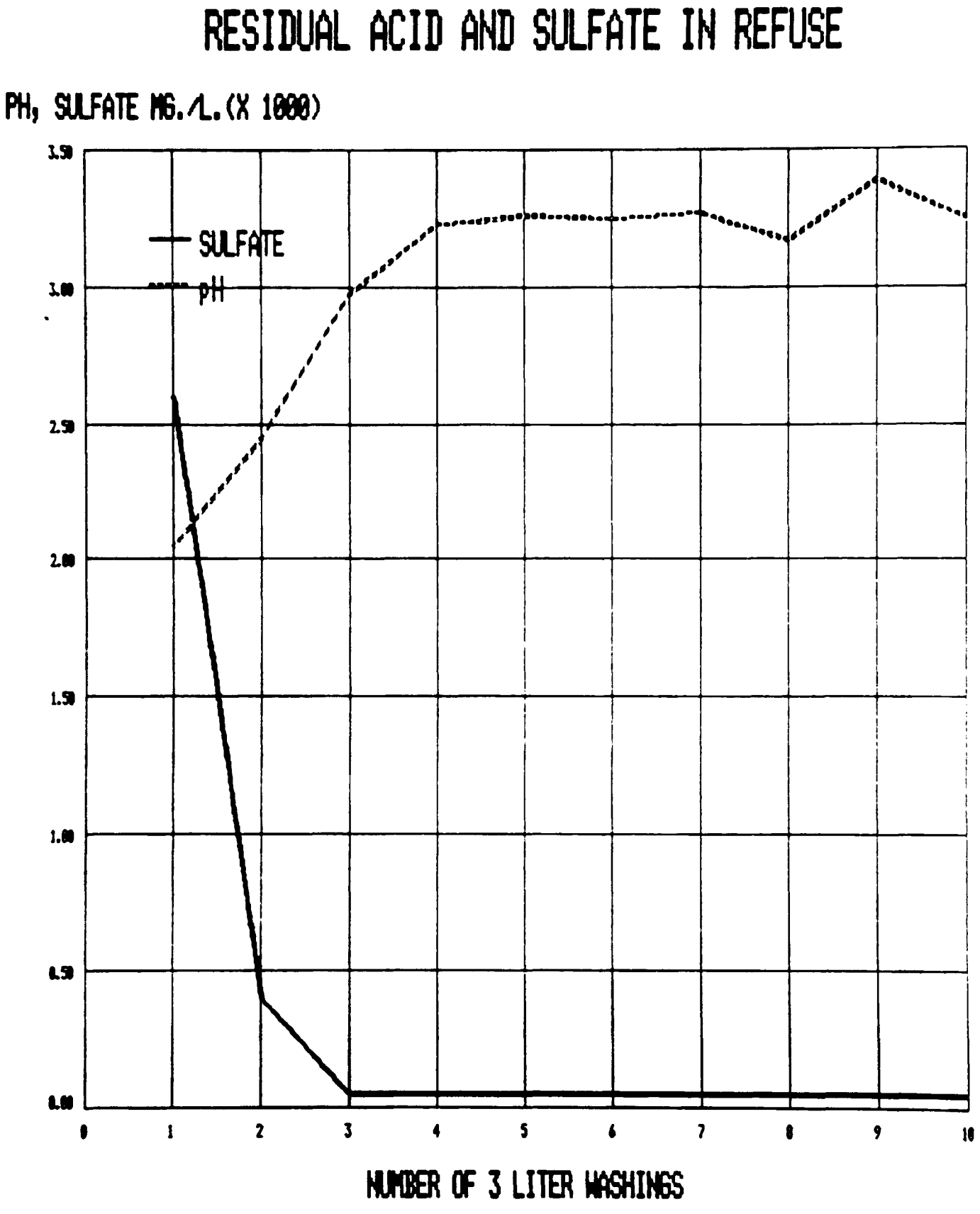

RESIDUAL ACID AND SULFATE IN REFUSE
PH, SULFATE MG./L.(X 1000)
SULFATE
pH
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0
1
2
3
4
5
6
7
8
9
10
NUMBER OF 3 LITER WASHINGS

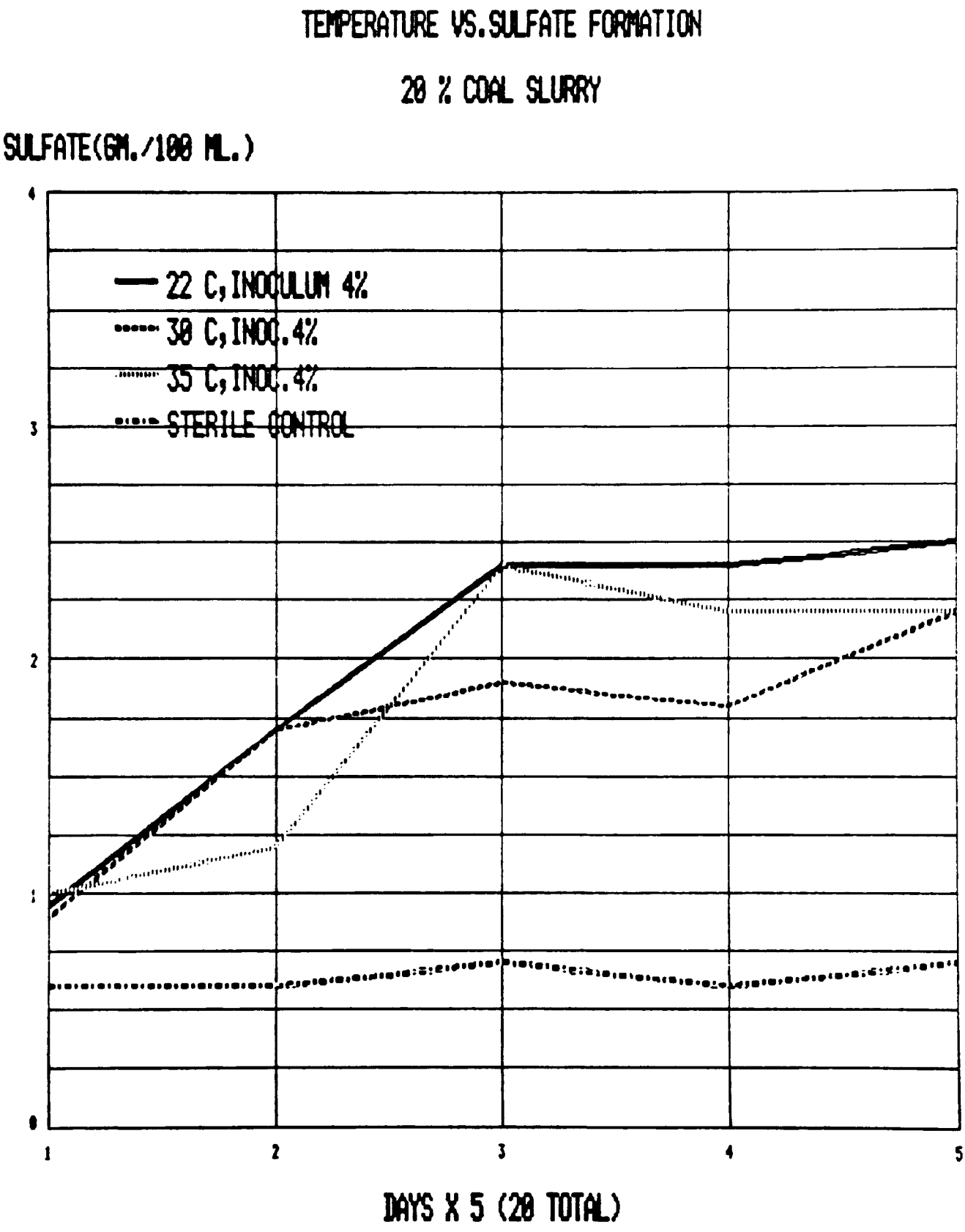
TABLE 1. pH and Sulfate Values of Selected Inhibitor Solutions Before and After Sterilization at 250 C, 20 min.

Inhibitor	Pre-Sterilization		Post-Sterilization	
	pH	SO_4	pH	SO_4
ABS 100 mg/L	4.9	99	4.2	100
LAS 100 mg/L.	7.5	100	6.2	100
Polyfon F 1%	9.3	82	9.3	73
Reax 81A 1%	8.7	88	8.7	86
Reax 88B 1%	4.2	89	4.8	65
Indulin C 1%	8.8	86	8.6	75

Effect of Incubation Temperature. Duplicate flasks containing 125 ml of a 20% slurry (wt. to vol.) of pulverized high sulfur coal (4.2%S) were inoculated with 5 ml. of an active enrichment of iron and sulfur oxidizing microorganism and incubated on a rotary shaker (180 rpm) at each of three temperatures: 22 +/- 2 C.,30 C., and 35 C. Acid and sulfate formation were sampled over a period of 20 days and compared to sterile control flasks which were not inoculated (5ml of sterile distilled water substituted).

Results of sulfate analysis vs time in days are presented in Figure 4 and show that 22 C. was optimum for sulfur oxidation in this test system. pH data showed no discernable difference amongst the three temperatures and the data are not plotted. On the basis of this experiment and previous information it was decided to conduct all subsequent experiments at ambient temperature (22 +/-2 C.).

Figure 4


TEMPERATURE VS.SULFATE FORMATION
20 % COAL SLURRY
SULFATE(GM./100 ML.)
22 C,INOCULUM 4%
30 C,INOC.4%
35 C,INOC.4%
STERILE CONTROL
0
1
2
3
4
1
2
3
4
5
DAYS X 5 (20 TOTAL)

pH Adjustment. On the basis of the above data it was anticipated that additions of test inhibitor solutions would alter the initial pH and sulfate concentrations in experimental flasks. Consequently, in subsequent flask experiments the pH of the refuse suspension was adjusted to within the range of 2.5 to 2.7 with dilute HCl after the addition of the 5 ml of inhibitor solution. The optimum pH for microbial pyrite oxidation is in the range 2 to 3. Variation in initial sulfate concentrations were recorded but not adjusted. No observable effect of initial pH adjustment on sample suspension could be detected.

Initial Inhibition Experiments

Initial experiments were conducted on 1300 gm. pulverized coal refuse which was washed with ten, 3 liter aliquots of distilled water and filtered through Whatman No. 10 filter paper. Washed refuse was resuspended to 20% (wt. to vol.) and 125 ml was dispensed into 250 ml Erlenmeyer flasks. The following inhibitors were added to yield the concentrations specified and the flasks were sterilized via autoclave: propionic acid, pyruvic acid four different formulations of lignin sulfonates produced by Westvaco (Polyfon F, Reax 81-A, Reax 88-B and Indulin -C). Stock solutions of the following substances were filter sterilized and added aeseptically to yield the specified concentrations: alkylbenzene sulfonate (ABS), linear alkylbenzene sulfonate (LAS), sodium lauryl sulfate (SLS), oxalic acid and sodium benzoate. Flasks were inoculated with 4 ml. of an enriched coal refuse culture that contained active iron and sulfur oxidizing organisms. The flasks were then incubated on a rotary shaker (180 rev. per min.) at 22 +/- 2 ^{o}C. Two ml samples were withdrawn periodically for pH and sulfate analysis and the volume in each flask was replaced with 2 ml. sterile distilled water.

Both inoculated (4 ml.) and sterile controls (i.e. no inhibitor added) were also maintained for comparative purposes.

Results

pH and sulfate formation (gm/100 ml. slurry) are plotted vs time in days over a 24 day period in the absence of added inhibitors and are presented in Figure 5. Effects of inhibitors are presented in Figures 6 through 11. ABS at 100 mg/L. completely inhibited acid and sulfate formation over the 24 day period whereas LAS at 100 mg/L. was ineffective.

Polyfon F at 0.1% was ineffective whereas Reax 81-A, Reax 88-B and Indulin-C each reduced the formation of acid and sulfate. However, 0.1% is a very high concentration for a potential inhibitor and no further experiments were conducted on this group of lignin sulfonates.

Benzoic acid, 0.14%; oxalic acid, 0.1%; pyruvic acid; and propionic acid 0.1% were completely effective at preventing sulfate and acid formation (i.e. values paralleled the sterile controls). Consequently no graphs have been included for this data. It was concluded that the concentrations of the above inhibitors (0.1%) was too high to be of practical use in the field.

Summary of Initial Experiments to Inhibit Acid and Sulfate Formation

20% coal refuse/water slurry

4% (5 ml.) pre-enriched inoculum

or 5 ml. sterile water in place of inoculum

Figure 5. Control (no additives) inoculated and sterile

Figure 6. ABS, 100 mg/L.

Figure 7. LAS, 100 mg/L.

Figure 8. Polyfon-F, 0.1%

Figure 9. Reax -81-A, 0.1%

Figure 10. Reax -88-B, 0.1%

Figure 11. Indulin -C, 0.1%

Benzoate	0.14%,	Complete inhibition,	No graph
Oxalate	0.1%,	Complete inhibition,	No graph
Propionate	0.1%,	Complete inhibition,	No graph
Pyruvate	0.1%,	Complete inhibition,	No graph

Figure 5

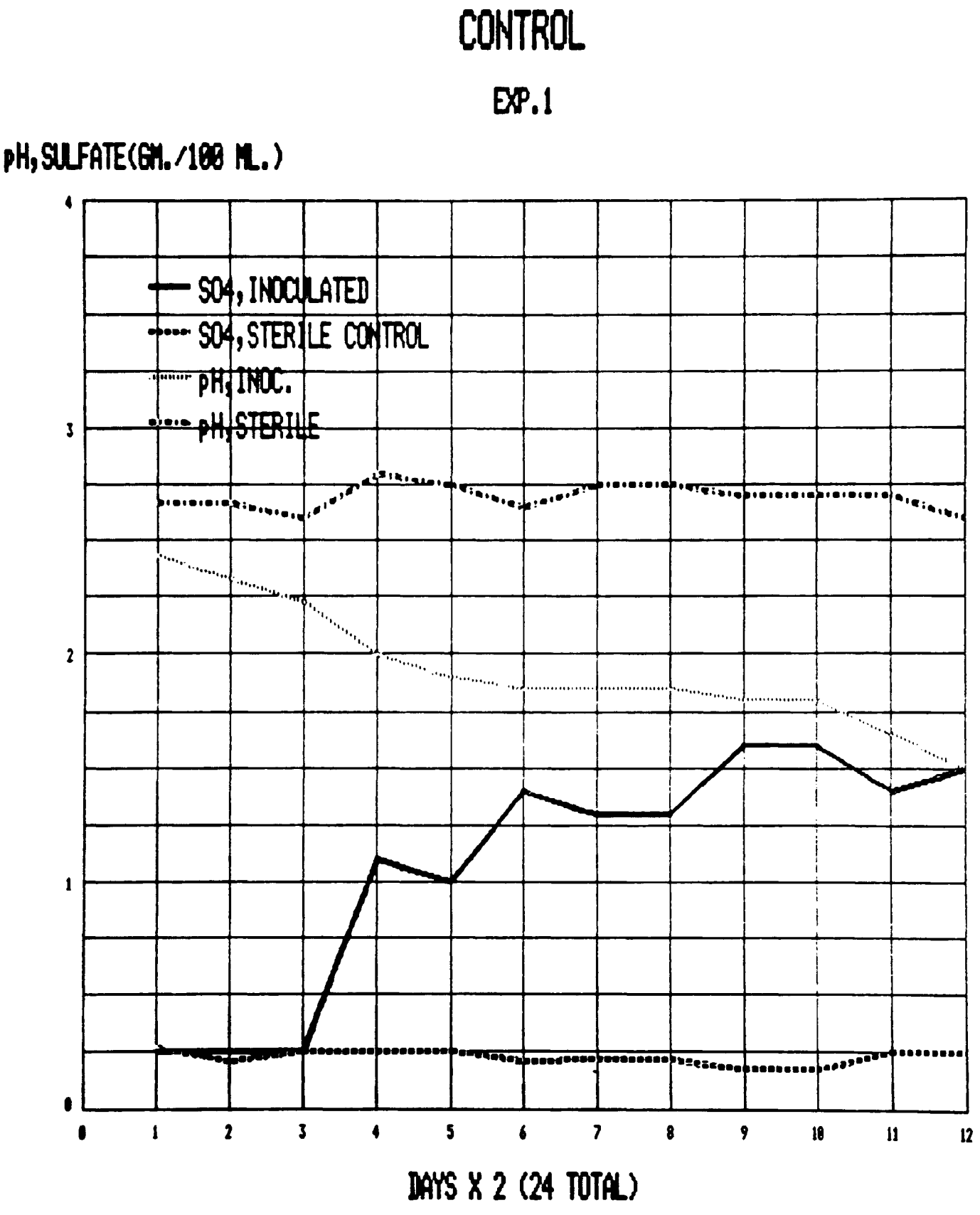

CONTROL
EXP.1
pH, SULFATE(GM./100 ML.)
SO4, INOCULATED
SO4, STERILE CONTROL
pH, INOC.
pH, STERILE
0
1
2
3
4
0 1 2 3 4 5 6 7 8 9 10 11 12
DAYS X 2 (24 TOTAL)

Figure 6

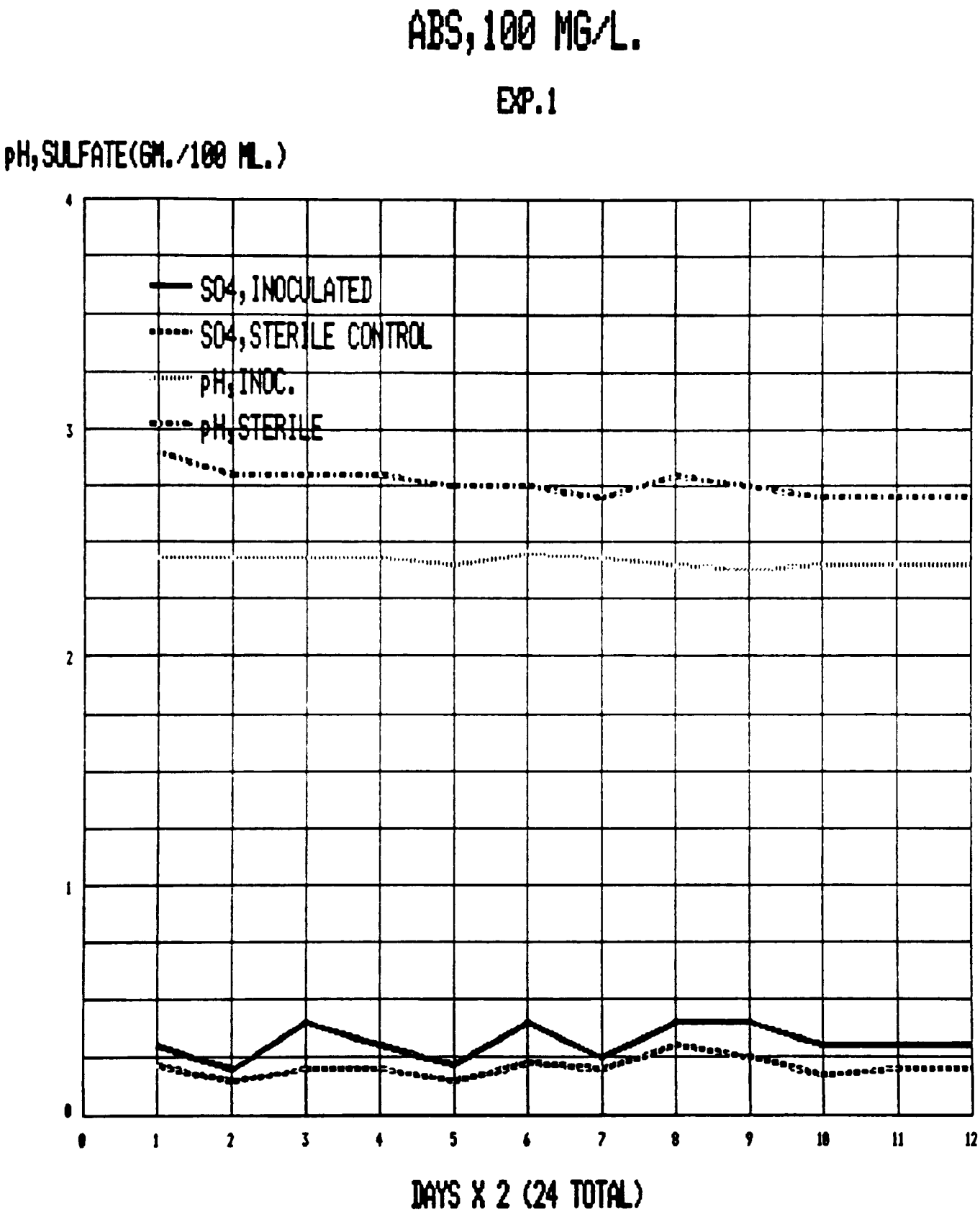
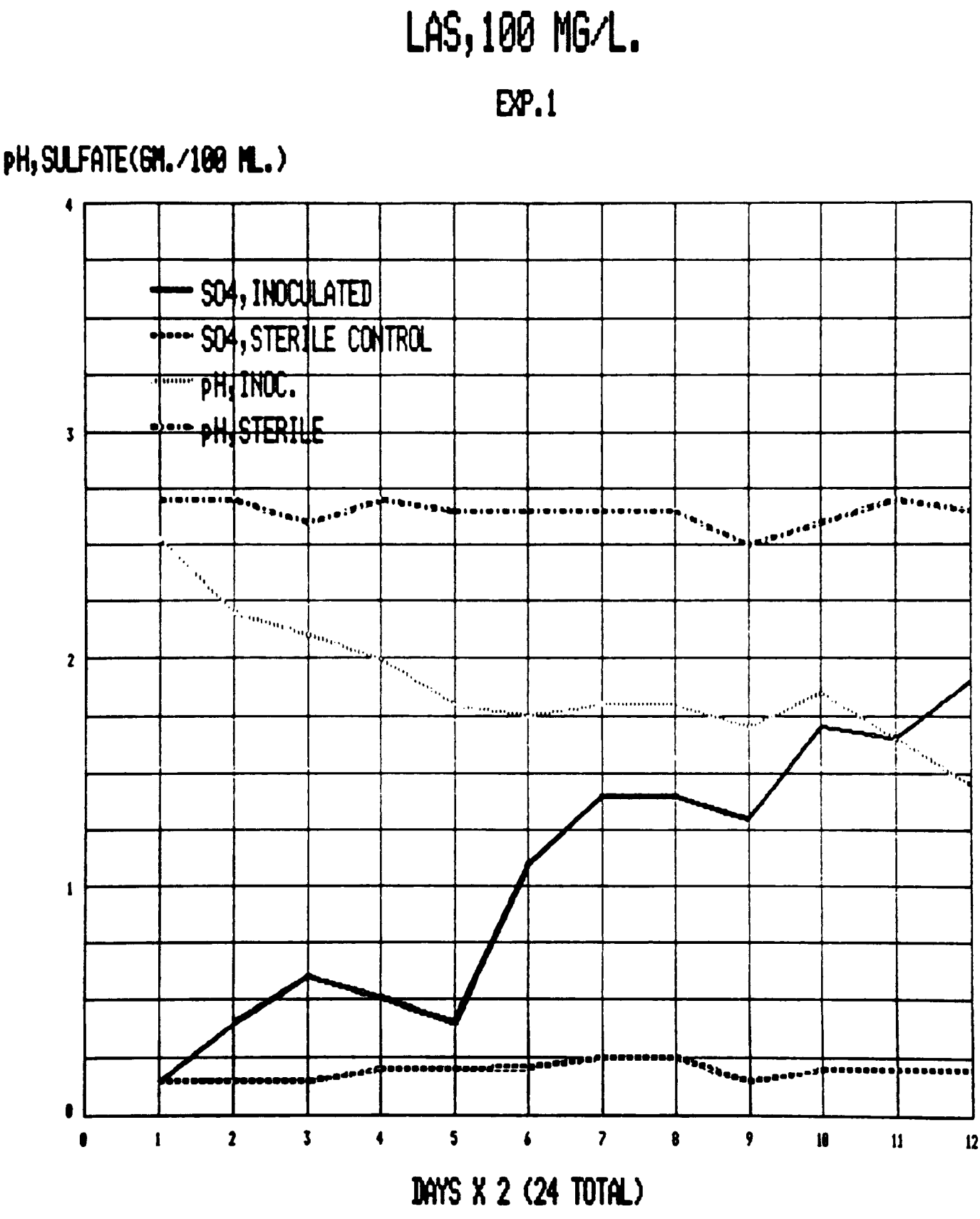
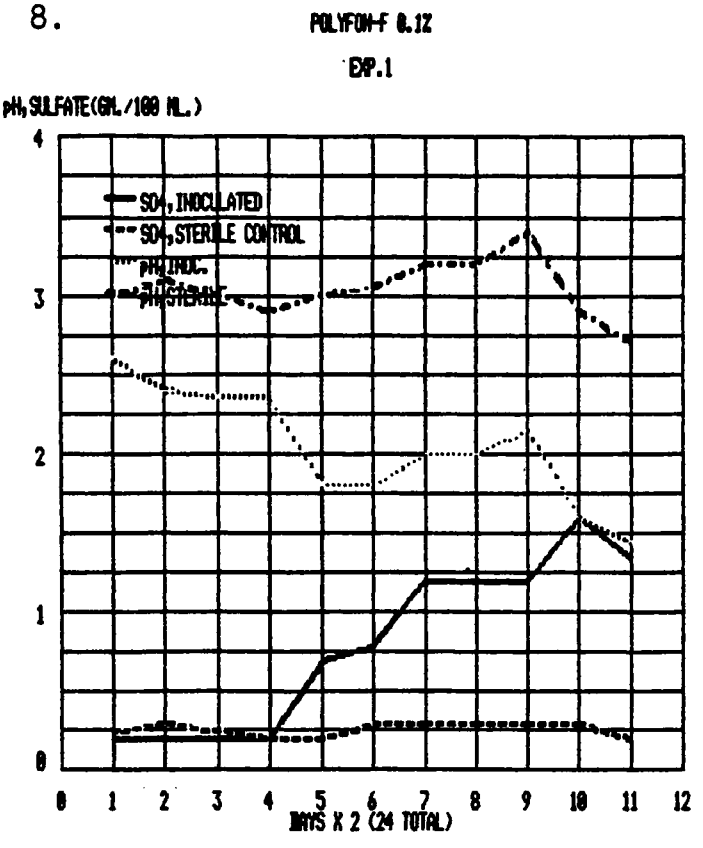

ABS,100 MG/L.
EXP.1
pH,SULFATE(GM./100 ML.)
SO4,INOCULATED
SO4,STERILE CONTROL
pH,INOC.
pH,STERILE
4
3
2
1
0
0
1
2
3
4
5
6
7
8
9
10
11
12
DAYS X 2 (24 TOTAL)

Figure 7

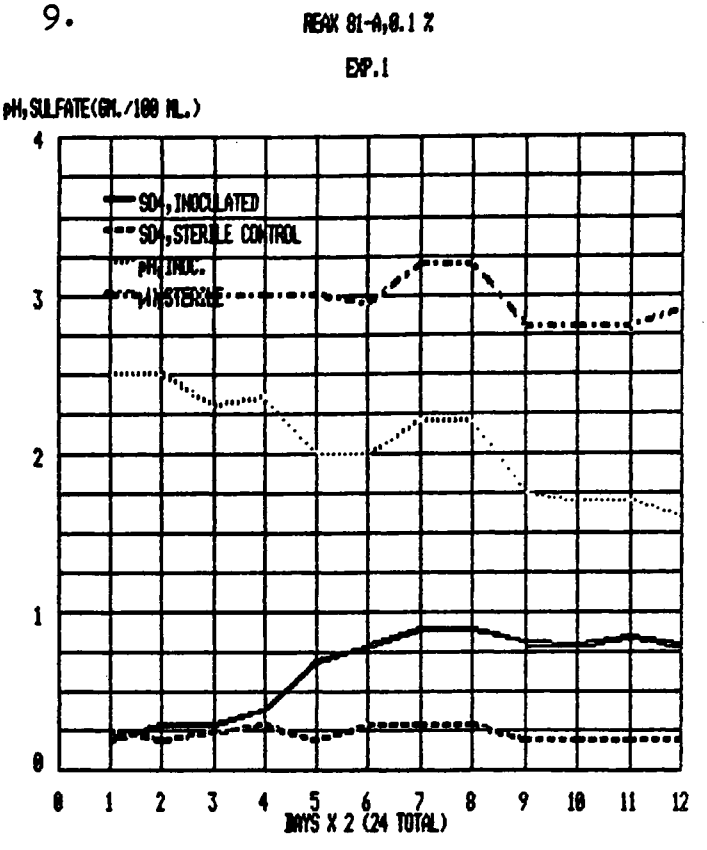

LAS,100 MG/L.
EXP.1
pH,SULFATE(GM./100 ML.)
SO4,INOCULATED
SO4,STERILE CONTROL
pH,INOC.
pH,STERILE
0
1
2
3
4
0
1
2
3
4
5
6
7
8
9
10
11
12
DAYS X 2 (24 TOTAL)

Figures 8,9,10, and 11

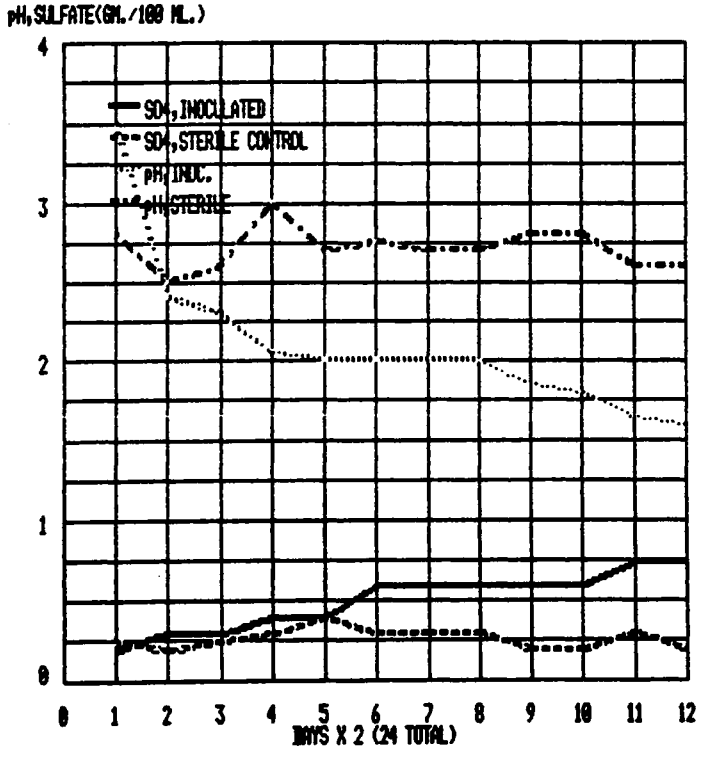
8.



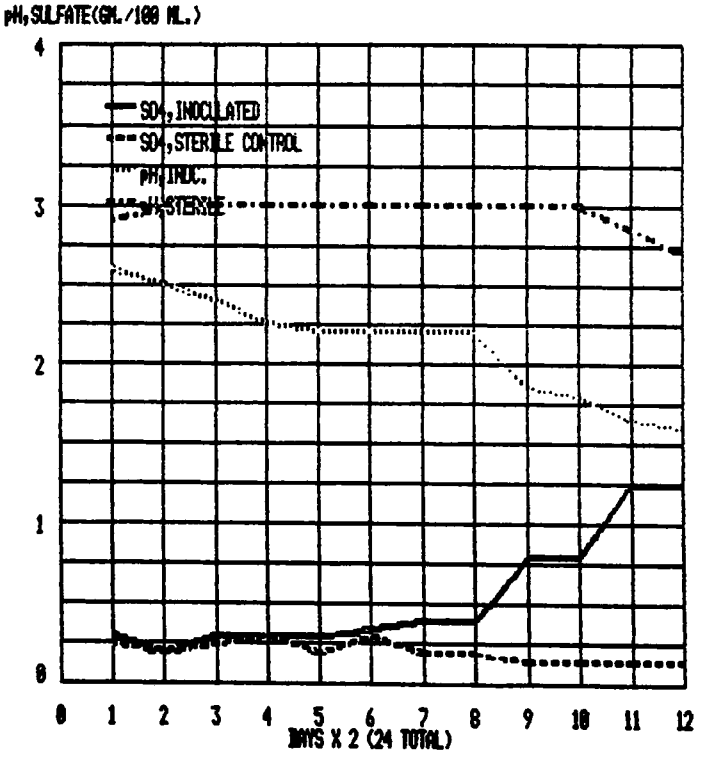
9.



10.



11.



Additional Inhibitor Experiments on 20% Coal Refuse

1200 g. pulverized refuse was washed with six,3 liter aliquots of distilled water, filtered and resuspended in distilled water to yield a 20% slurry (final pH 3.2). The slurry was mixed at 400 rpm for 14 hours in a Microferm fermenter jar (pH 3.2), then 125 ml aliquots were dispensed into 250 ml. Erlenmeyer flasks and sterilized by autoclave. Chemical inhibitors were sterilized separately by autoclave after adjusting pH to the range 2.0 to 3.0 where necessary by addition of 10 N H_2SO_4 as shown in Table 2.

TABLE 2.

Stock Solution	pH	Adjusted to pH	10N H_2SO_4
propionic acid	5.8	2.2	1.5 ml.
pyruvic acid	4.2	2.0	1.0 ml.
ABS	3.0	not adjusted	
LAS	2.7	not adjusted	
SLS	2.85	not adjusted	
formic acid	2.2	not adjusted	
hexanoic acid	2.3	not adjusted	

Inhibitors were added to flasks to yield the concentrations indicated, inoculated with 5 ml. (4%) of a pre-enriched active culture of iron and sulfur oxidizers, then incubated at 22 +/- 2 ^{o}C. on a rotary shaker at 180 rpm.

Results

Figure 12 shows the effect of 100 mg./l. concentrations of SLS, ABS and LAS compared to the inoculated control without added inhibitor. ABS was somewhat effective and SLS was the most effective at both decreasing the total amount of pyrite oxidized to sulfate and in delaying the onset of oxidation i.e. there was no evidence of oxidation until the fourth week in the presence of SLS.

pH values for SLS, ABS and LAS are presented in Figure 13. pH in the presence of LAS paralleled the control indicating that LAS was ineffective in preventing pyrite oxidation under the experimental conditions, whereas both ABS and SLS prevented acid formation and the pH values were generally consistent with the sulfate formation values.

Figure 14 presents similar data in the presence of 0.1% concentrations of propionic, pyruvic, formic and hexanoic acids. These organic acids were all inhibitory to pyrite oxidation. Formic was the most effective followed by hexanoic, propionic and pyruvic as compared to the uninhibited control. pH data for the above ewperiments is presented in Figure 15 and are in agreement with sulfate formation data. i.e. each of the organic acids prevented acid formation with effectiveness in the sequence: formic > hexanoic > propionic > pyruvic. However, 0.1% concentrations are too high for field use.

Summary of Experiments

20% coal refuse slurry, 125 ml./250 ml. flask

4% inoculum of pre-enriched iron and sulfur oxidizers

22 +/- 2 °C., 28 days.

Figure 12. Sulfate formation vs time (4 weeks) in presence of SLS, LAS, ABS, each at 100 mg./L.

Figure 13. pH change vs time (4 weeks) in presence of SLS, LAS, ABS each at 100 mg./L.

Figure 14. Sulfate formation vs time (4 weeks) in presence of propionic, pyruvic, formic and hexanoic acids, each at 0.1% concentration.

Figure 15. pH change vs time (4 weeks) in presence of propionic, pyruvic, formic and hexanoic acids each at 0.1% concentration.

Figure 12

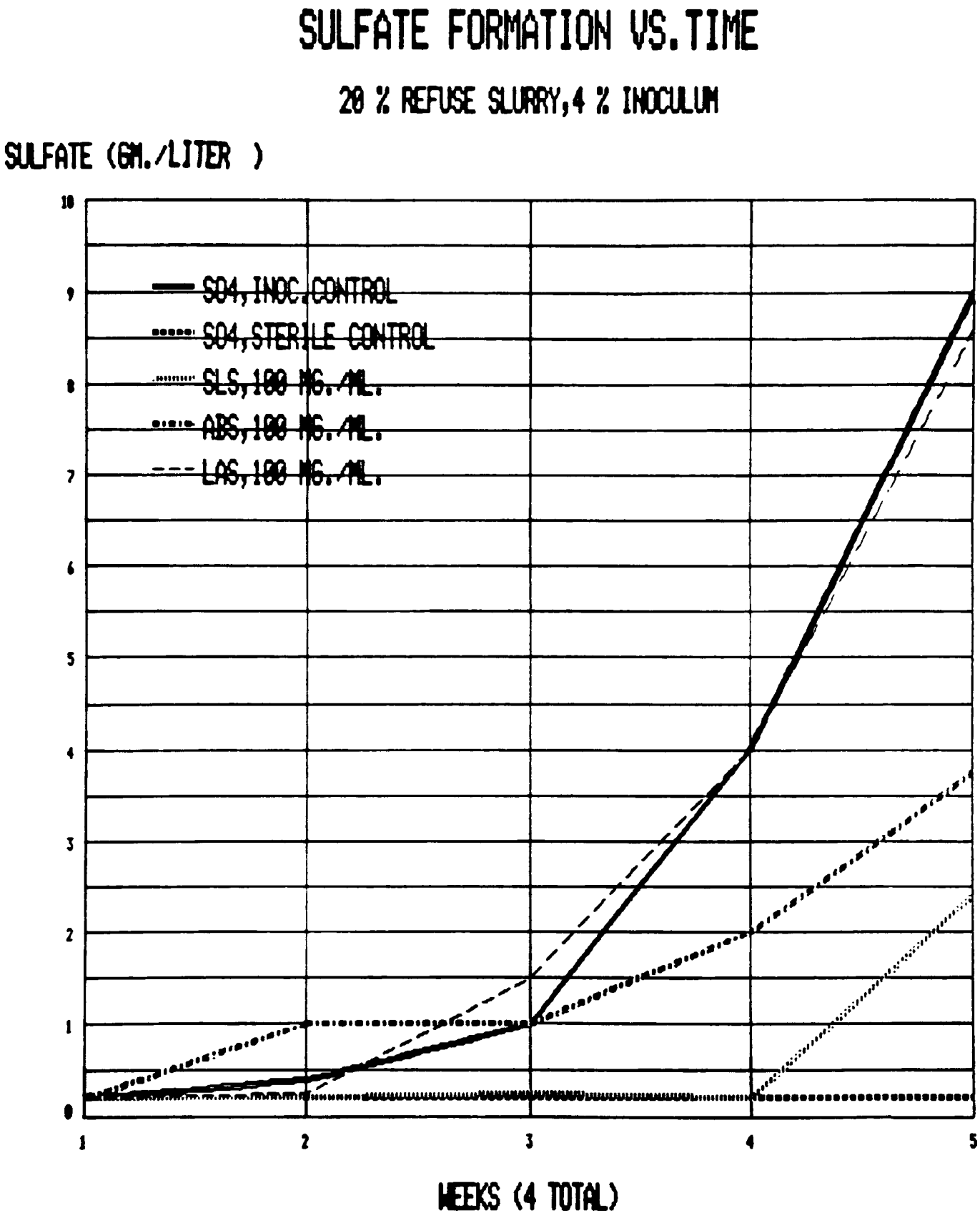

SULFATE FORMATION VS.TIME
20 % REFUSE SLURRY,4 % INOCULUM
SULFATE (GM./LITER)
SO4,INOC.CONTROL
SO4,STERILE CONTROL
SLS,100 MG./ML.
ABS,100 MG./ML.
LAS,100 MG./ML.
0
1
2
3
4
5
6
7
8
9
10
1
2
3
4
5
WEEKS (4 TOTAL)

Figure 13

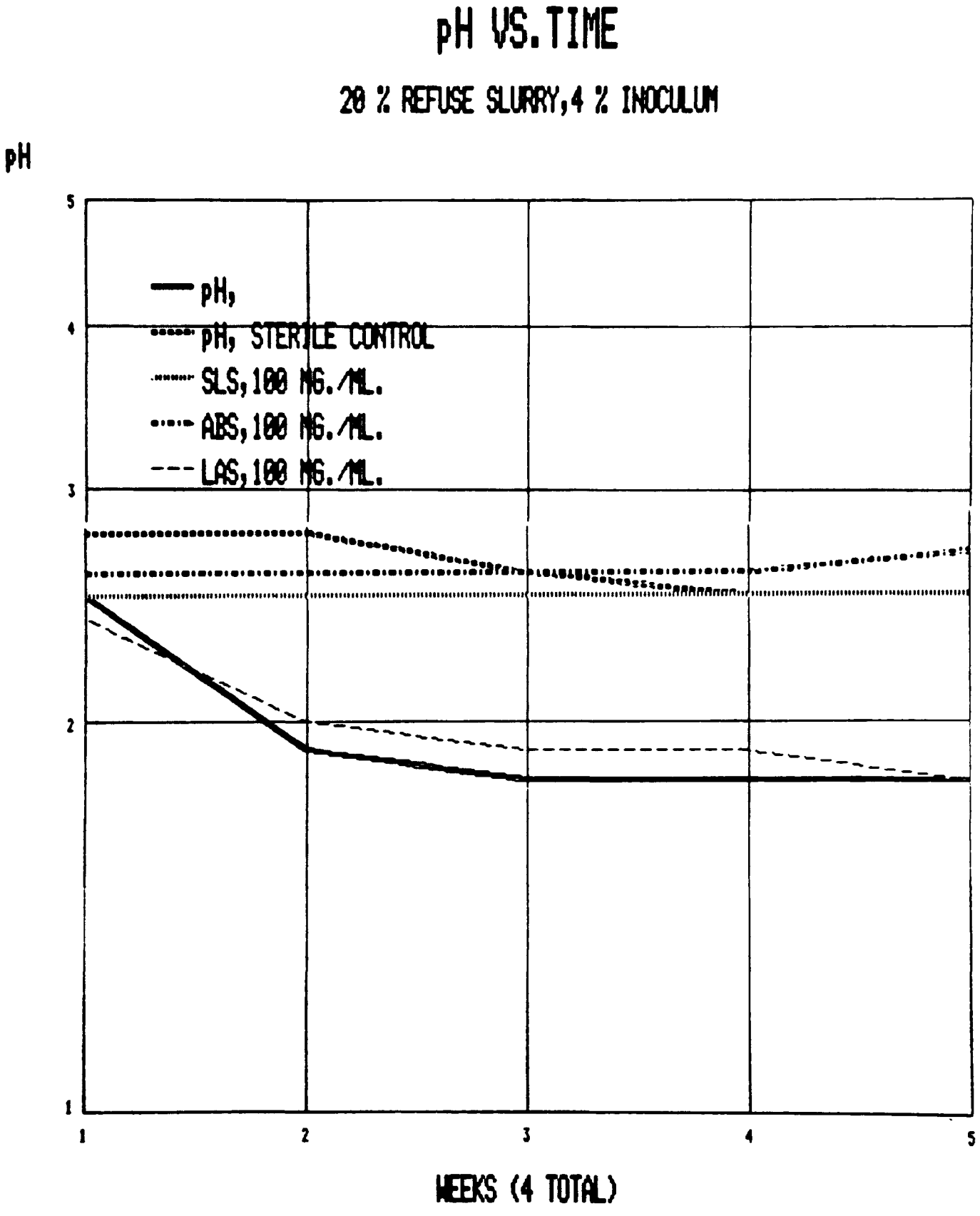

pH VS. TIME
20 % REFUSE SLURRY, 4 % INOCULUM
pH
5
4
3
2
1
pH,
pH, STERILE CONTROL
SLS, 100 MG./ML.
ABS, 100 MG./ML.
LAS, 100 MG./ML.
1
2
3
4
5
WEEKS (4 TOTAL)

Figure 14

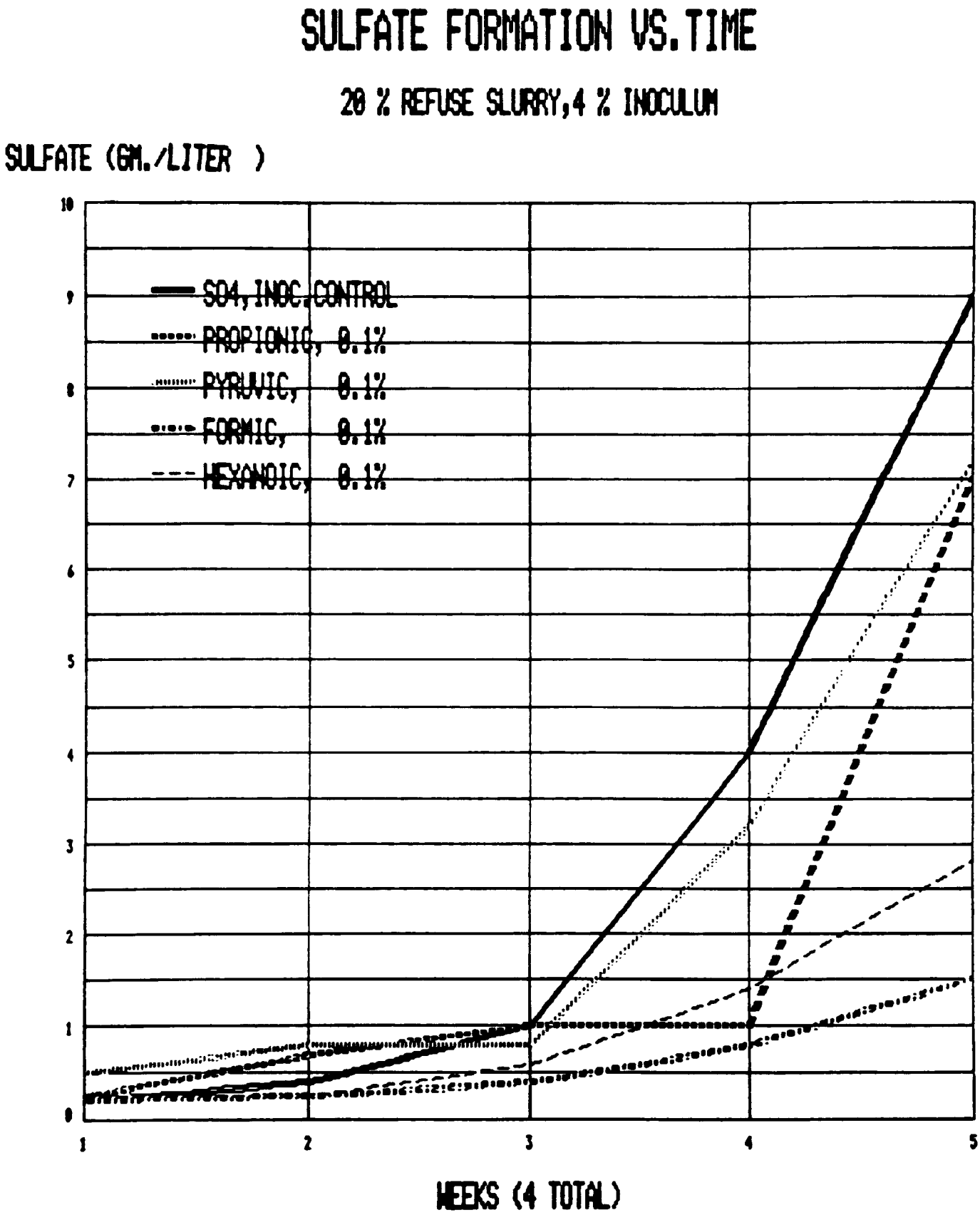
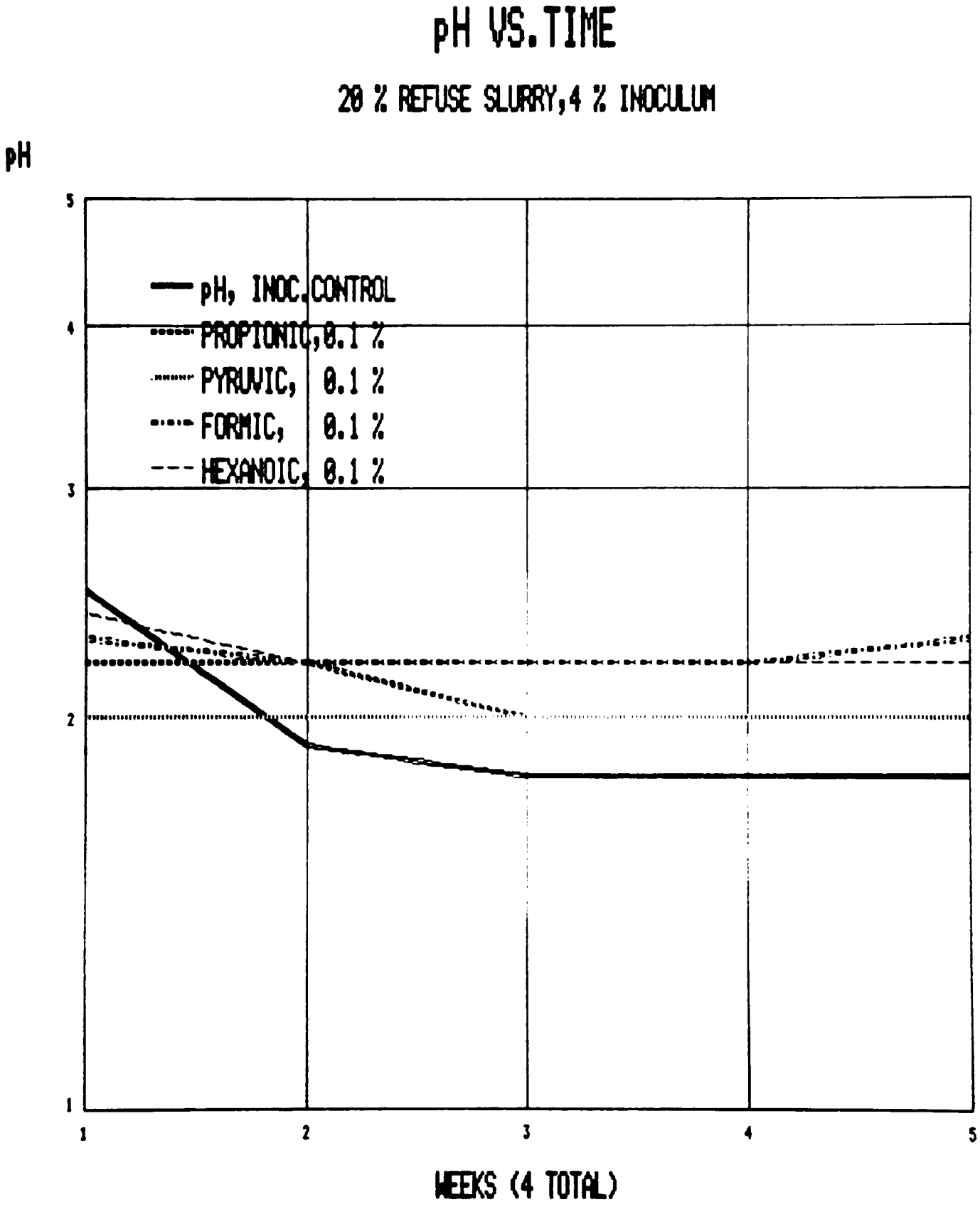

SULFATE FORMATION VS. TIME
20 % REFUSE SLURRY, 4 % INOCULUM
SULFATE (GM./LITER)
SO4, INOC. CONTROL
PROPIONIC, 0.1%
PYRUVIC, 0.1%
FORMIC, 0.1%
HEXANOIC, 0.1%
0
1
2
3
4
5
6
7
8
9
10
1
2
3
4
5
WEEKS (4 TOTAL)

Figure 15


pH VS.TIME
20 % REFUSE SLURRY,4 % INOCULUM
pH
5
4
3
2
1
pH, INOC.CONTROL
PROPIONIC,0.1 %
PYRUVIC, 0.1 %
FORMIC, 0.1 %
HEXANOIC, 0.1 %
1
2
3
4
5
WEEKS (4 TOTAL)

Inhibition Experiments in 30% Coal Refuse, 1% Inoculum.

Earlier experiments indicated that the anionic detergents, SLS and ABS were the most effective inhibitors evaluated with respect to concentration relative to inhibition of thiobacilli, pyrite oxidation, iron and/or sulfur oxidation.

In order to be effective in the field, the chemicals must be inhibitory in the presence of a high solids content. i.e. refuse piles are not slurries. Further, in the case of ABS, it is known that the effective concentration of ABS is directly related to the number of organisms per ml. to be inhibited (see Figure 2). Those evaluations were made in the absence of suspended coal and mineral particulates.

Consequently, it was decided to increase the slurry concentration from 20% to 30% and to reduce the inoculum from 4% to 1% (by volume) in order to further evaluate the effectiveness of SLS, ABS, LAS and Benzoic Acid and to begin to more closely approximate field situations. New refuse was obtained from Peabody, Sunny-Hill Mine for those experiments and processed in the laboratory as previously described. Benzoic acid was selected because it is an effective inhibitor of many heterotrophic microorganisms which are known to indirectly stimulate autotrophic pyrite oxidation (21) and because it is relatively inexpensive and non-toxic to higher forms of life. It has FDA clearance as a food additive for himan consumption (as is the case for several of the other inhibitors tested).

Results

ABS and LAS were ineffective inhibitors of sulfate formation in the concentrations evaluated (5, 10, and 20 mg. per L.) under the experimental conditions as presented in Figures 16 and 17. Both SLS and Benzoic Acid (Figures 18 and 19) appeared to slightly inhibit sulfate formation at the 20 mg. per ml. concentration, but not at the lower concentrations. Actually, lower concentrations of SLS, ABS and LAS (5 and 10 mg./L.) all appeared to stimulate the rate of pyrite oxidation compared to controls for the first 10 days of the experiment. After 12 days the total sulfate formation in the controls exceeded that of the test inhibitors in all cases. Benzoic acid and SLS at 20 mg./L. also appeared to retard acid formation; where as ABS and LAS at 20 mg./L. did not as shown in Figure 20.

Summary of Inhibition Experiments on 30% Coal Refuse, 1% Inoculum

30% Coal Refuse, 125 ml. per 250 ml. flask

1% Inoculum of pre enriched iron and sulfur oxidizers

22 +/- 2 C., 12 days

Inhibitors: ABS, LAS, SLS, Benzoic Acid

Concentrations: 0, 5, 10, 20, mg./ml.

Figure 16. Sulfate formation vs time (12d)
in presence of ABS (0, 5, 10, 20 mg./ml.)

Figure 17. Sulfate formation vs time (12 d)
in presence of LAS (0, 5, 10, 20 mg./ml.)

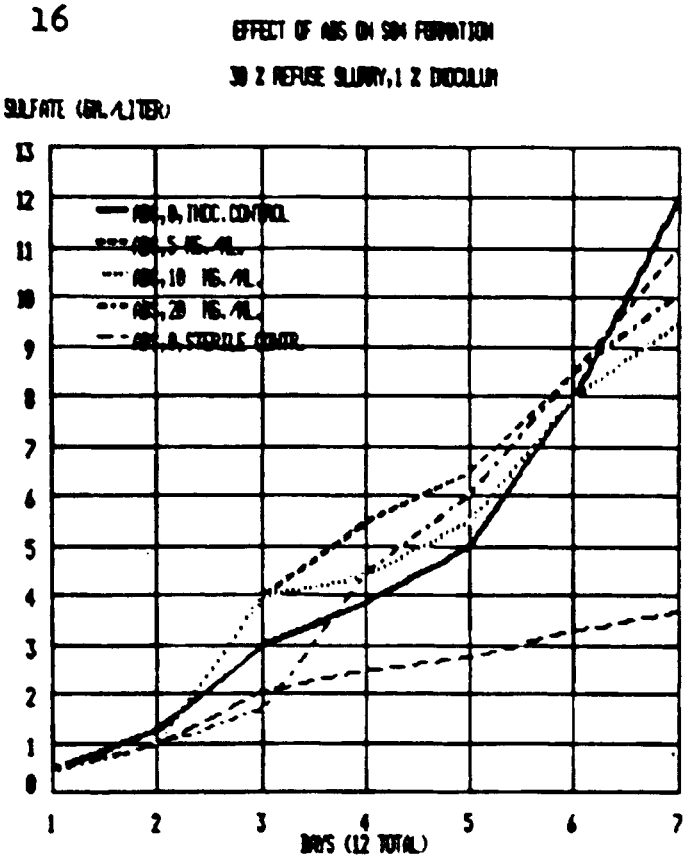
Figure 18. Sulfate formation vs time (12d)
in presence of SLS (0, 5, 10, 20 mg./ml.)

Figure 19. Sulfate formation vs time (12 d)
in presence of Benzoic Acid (0, 5, 10, 20 mg./ml.)

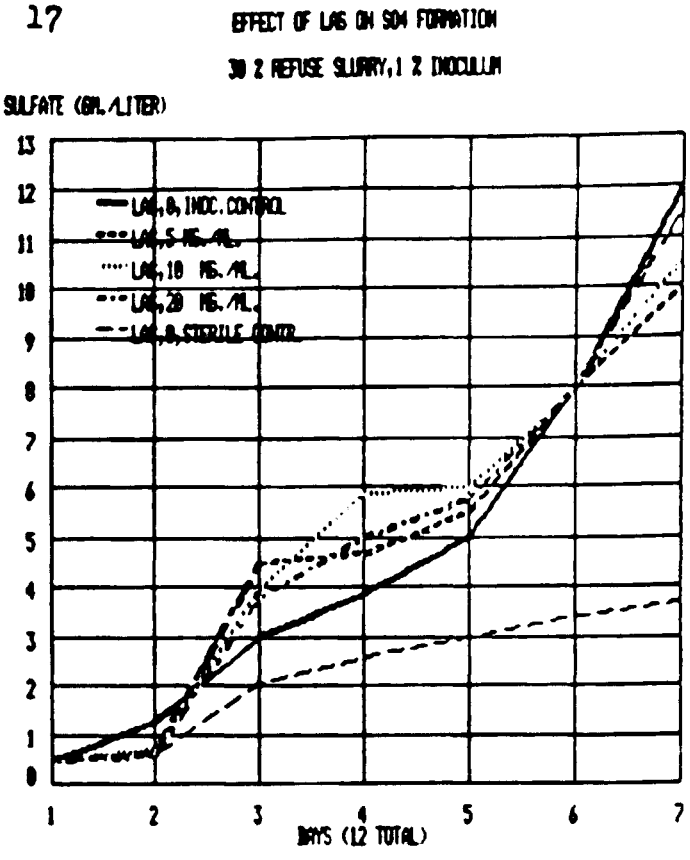
Figure 20. pH change vs time (12d)
in the presence of ABS, LAS, SLS and Benzoic Acid
each at 20 mg./ml.

Figures 16,17,18 and 19

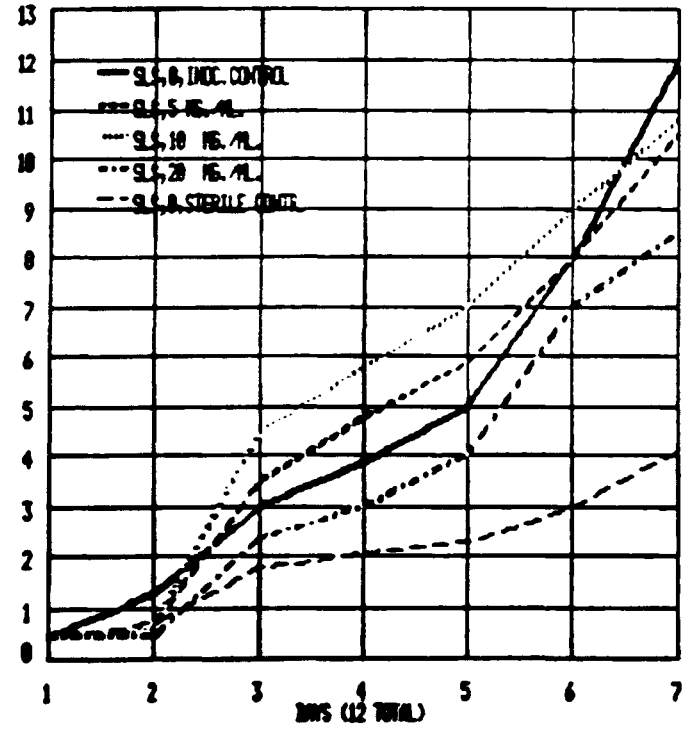
16



17



18



19

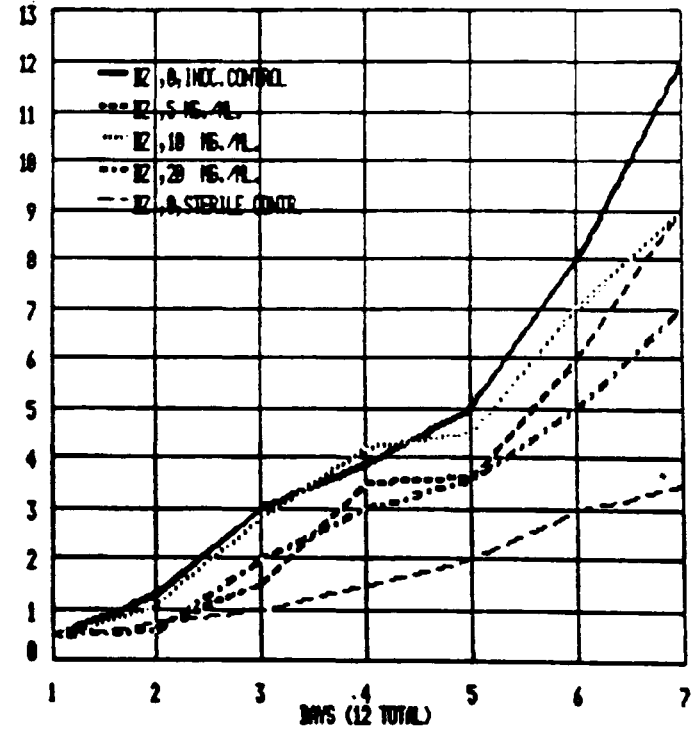
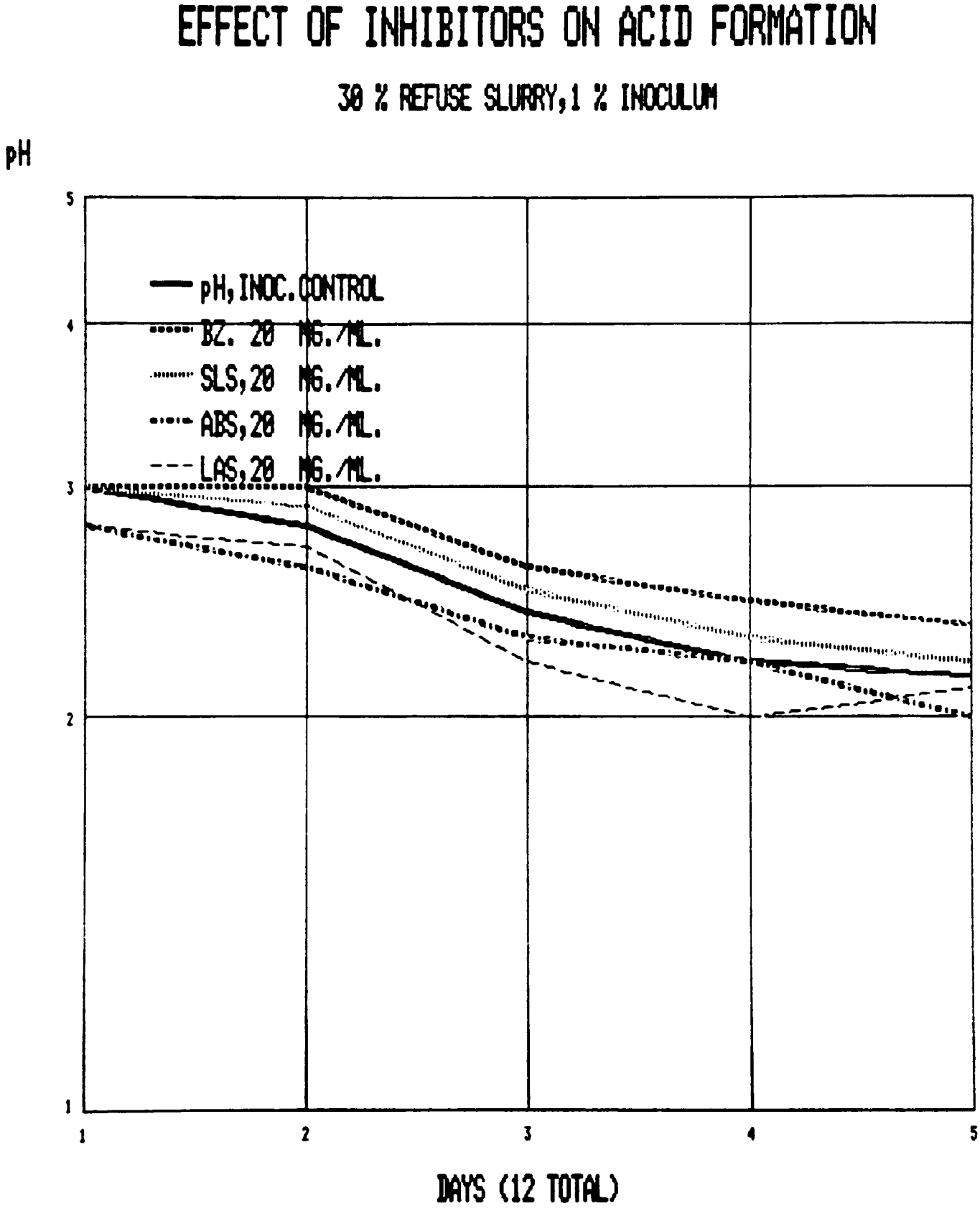


Figure 20


EFFECT OF INHIBITORS ON ACID FORMATION
30 % REFUSE SLURRY,1 % INOCULUM
pH
5
4
3
2
1
pH,INOC.CONTROL
BZ. 20 MG./ML.
SLS,20 MG./ML.
ABS,20 MG./ML.
LAS,20 MG./ML.
1
2
3
4
5
DAYS (12 TOTAL)

Additional Experiments on 30% Coal Refuse, 1% Inoculum

This series of experiments examined increased concentration of the inhibitors: ABS, SLS and Benzoic Acid (each at 25, 50, 75 and 100 mg./L.) compared to the previous experiments in 30% refuse slurry with 1% inoculum of pre-enriched iron and sulfur oxidizers. In addition, combinations of Benzoic Acid plus ABS and Benzoic Acid plus SLS were utilized.

Results

Sulfate formation and pH change over a 26 day period for both inoculated and sterile controls (no inhibitor added) are presented in Figure 21. In Figure 22 which plots changes in sulfate formation in the presence of ABS, it can be seen that 25 mg ABS per liter actually stimulated pyrite oxidation, whereas the higher concentrations (50, 75, 100 mg./L.) reduced pyrite oxidation generally in amounts related to concentration of ABS. Figure 23 shows pH change for the same concentrations of ABS presented in Figure 22 over the 26 day period. Although the daily data vary considerably, the general tendency indicates that ABS did not reliably prevent acid formation in this concentration range.

The effect of SLS on sulfate production in the same concentration range is shown in Figure 24. All concentrations of SLS reduced sulfate production for the first 12 days. However after 12 days the 75 mg.SLS/L. stimulated sulfate formation. No explanation of this is available, in comparison to the 25 and 50 mg/L. concentrations which continued to reduce sulfate formation over the 26 day period when compared to the control sample. 100 mg SLS per L. completely inhibited sulfate formation for the first 12 days and significantly reduced it for the remaining 14 days.

Benzoic acid was an effective inhibitor at all concentrations and its effectiveness was directly related to concentration for the first 18 days. During the period between days 18 and 26 the 75 mg./L. concentration nearly completely inhibited sulfate formation, whereas the 100 mg./L sample allowed some pyrite oxidation. Such variability relative to concentration during later stages of experiments is likely attributable to variation in coal refuse material.

Figure 26 plots the effect of a combination of benzoic acid plus ABS (each at 25, 50, 75 and 100 mg./L.) on sulfate formation. In general the 25 and 50 mg./L. combinations were not effective inhibitors beyond 12 to 14 days, whereas the 75 mg./L. concentration extended effectiveness to 20 days and 100 mg./L. was quite effective throughout the 26 day experiment.

The combination of benzoic acid plus SLS in the same concentration range is presented in Figure 27. All concentrations of benzoic and SLS reduced pyrite oxidation over the 26 days and generally in proportion to inhibitor concentration. 100mg./L. was very effective at inhibiting sulfate formation.

The pH change in the presence of SLS and Benzoic acid at 100 mg./L. and of the combinations: Benzoic plus ABS and Benzoic plus SLS each at 100 mg./L. are chartec in Figure 28. All reduced acid formation over the 26 days with the exception of benzoic acid alone during the latter days of the experiment with the sulfate formation data. No plots of pH vs time for concentrations lower than 100 mg./L. are included because they did not clearly illustrate trends.

Summary of Experiments

30% coal refuse slurry, 125 ml./250 ml. flask

1% inoculum of pre-enriched iron and sulfur oxidizers

22 +/- 2 °C., 26 days.

Figure 21. Inoculated and Sterile Controls (no inhibitors).

Figure 22. Sulfate formation in presence of ABS at 25, 50, 75, 100 mg./L.

Figure 23. pH change in presence of ABS at 25, 50, 75, 100 mg./L.

Figure 24. Sulfate formation in presence of SLS at 25, 50, 75, 100 mg./L.

Figure 25. Sulfate formation in presence of Benzoate at 25, 50, 75, 100 mg./L.

Figure 26. Sulfate formation in presence of Benzoate plus ABS, each at 25, 50, 75, snf 100 mg./L.

Figure 27. Sulfate formation in presence of Benzoate plus SLS, each at 25, 50, 75, 100 mg./L.

Figure 28. pH change in presence of SLS, Benzoate, Benzoate plus ABS and Benzoate plus SLS each at 100 mg./L.

Figure 21

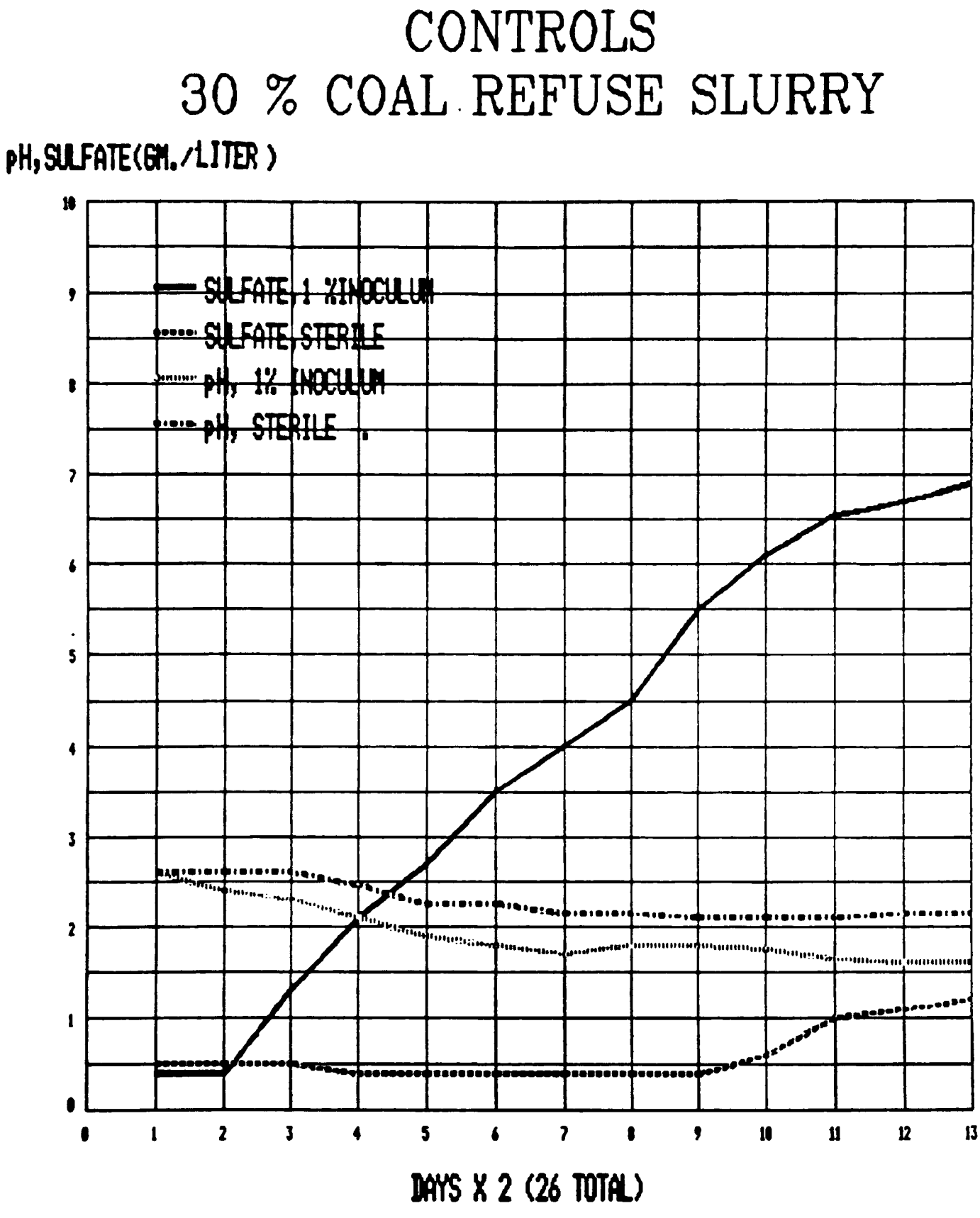

CONTROLS
30 % COAL REFUSE SLURRY
pH, SULFATE(GM./LITER)
SULFATE, 1 %INOCULUM
SULFATE, STERILE
pH, 1% INOCULUM
pH, STERILE
10
9
8
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
DAYS X 2 (26 TOTAL)

Figure 22

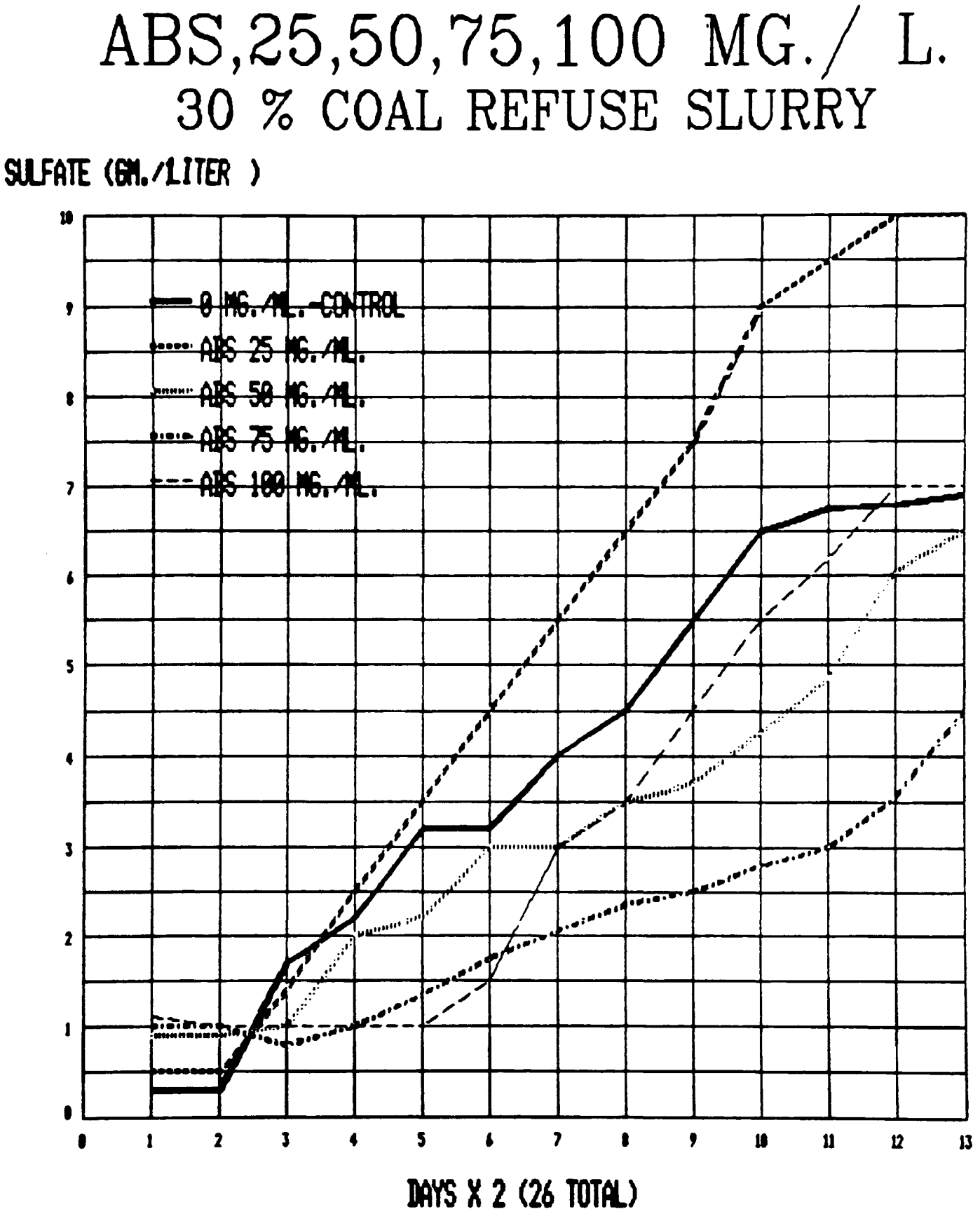

ABS,25,50,75,100 MG./ L.
30 % COAL REFUSE SLURRY
SULFATE (GM./LITER)
0 MG./ML.-CONTROL
ABS 25 MG./ML.
ABS 50 MG./ML.
ABS 75 MG./ML.
ABS 100 MG./ML.
0
1
2
3
4
5
6
7
8
9
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
DAYS X 2 (26 TOTAL)

Figure 23

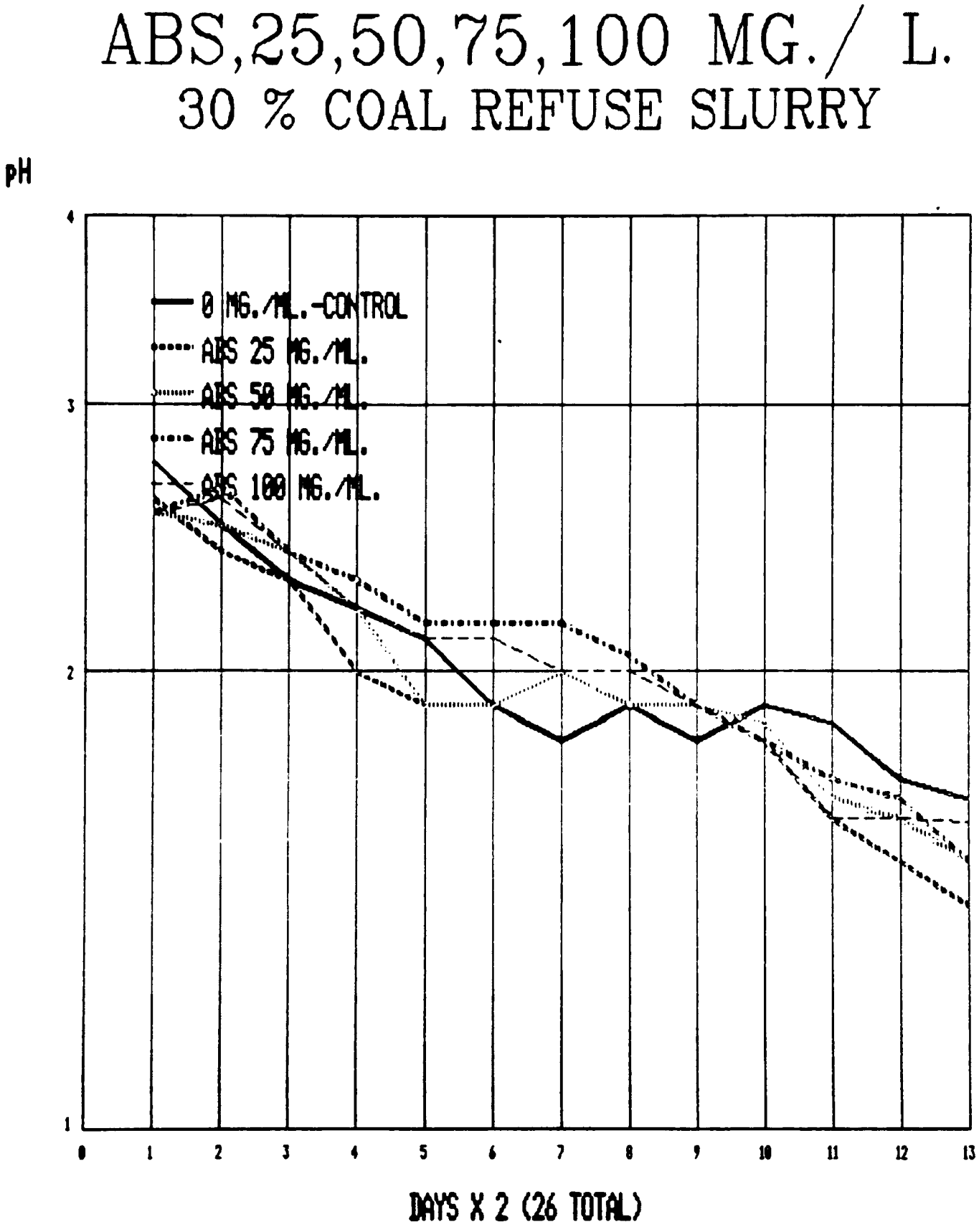

ABS,25,50,75,100 MG./ L.
30 % COAL REFUSE SLURRY
pH
0 MG./ML.-CONTROL
ABS 25 MG./ML.
ABS 50 MG./ML.
ABS 75 MG./ML.
ABS 100 MG./ML.
4
3
2
1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
DAYS X 2 (26 TOTAL)

Figure 24

SLS,25,50,75,100 MG./ L.
30 % COAL REFUSE SLURRY

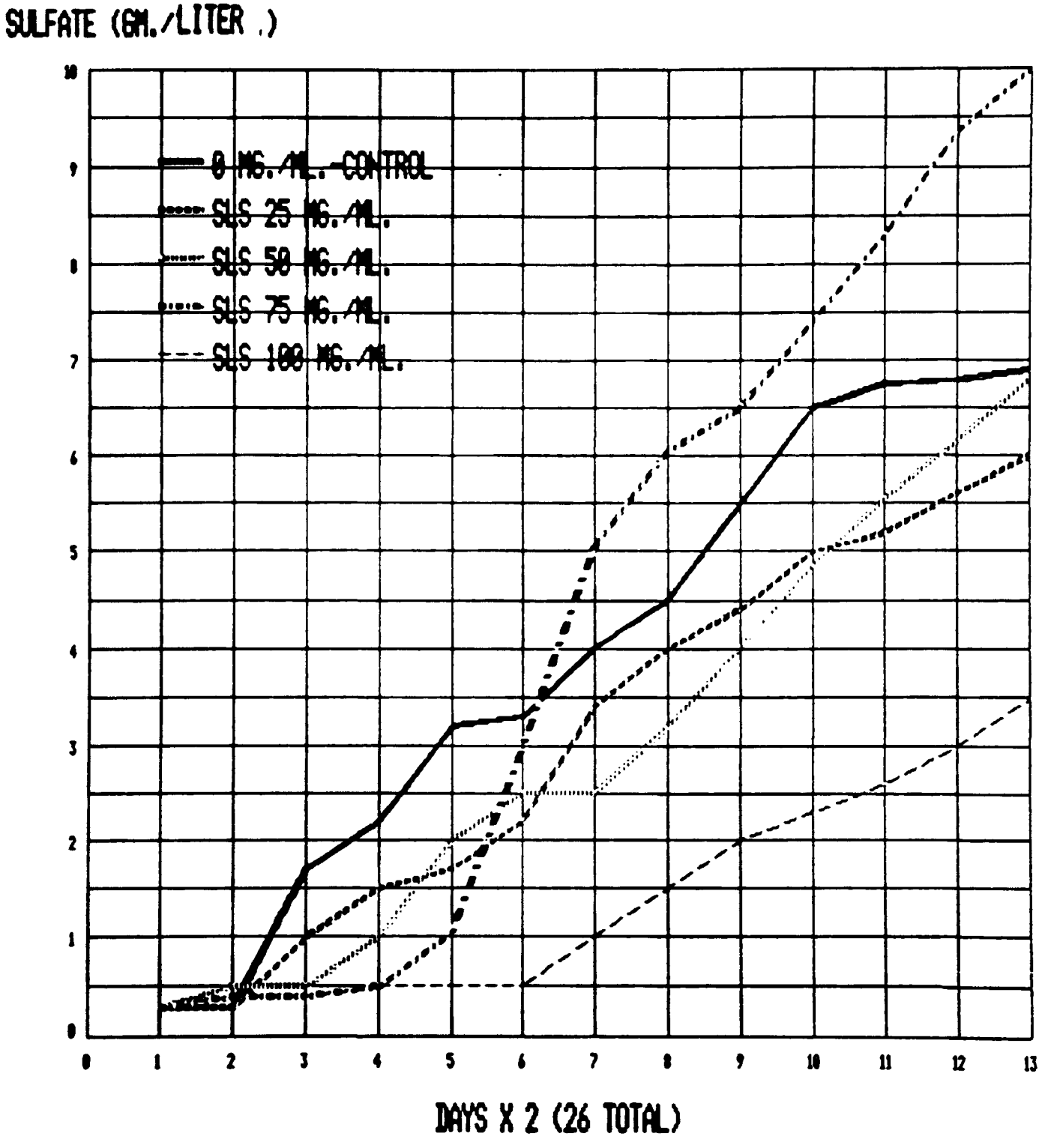


Figure 25

BENZOATE,25,50,75,100 MG./L.
30 % COAL REFUSE SLURRY

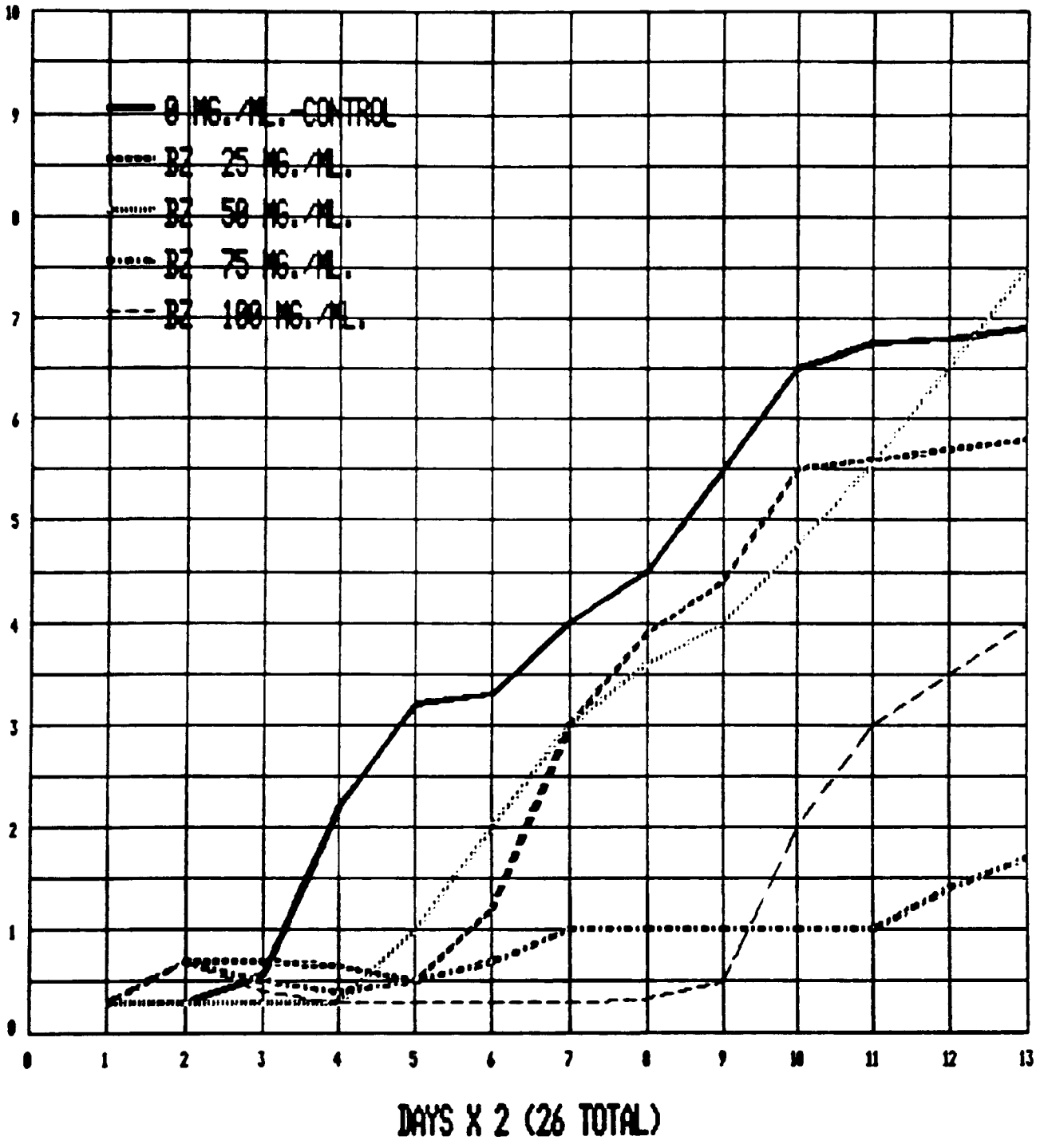


Figure 26

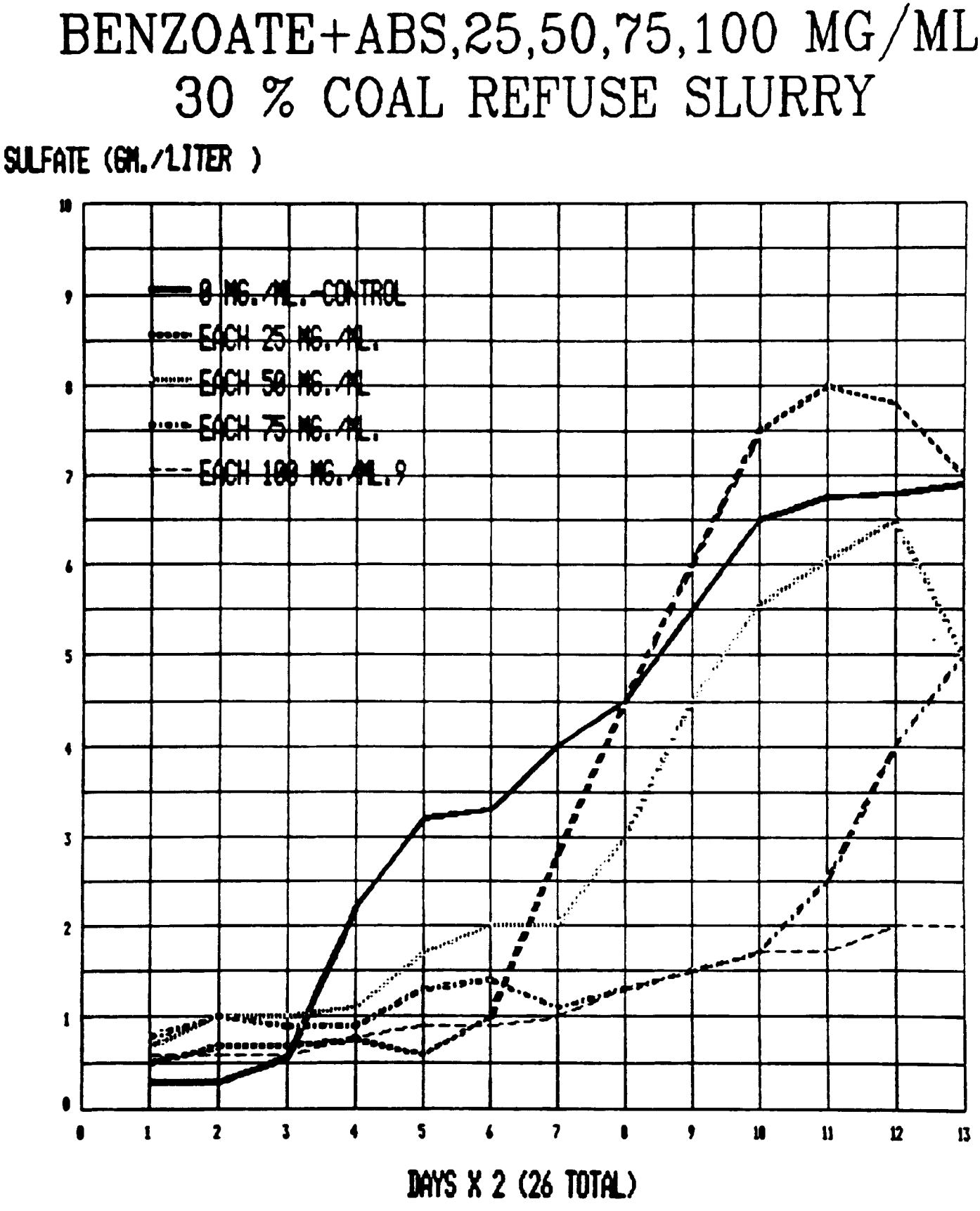

BENZOATE+ABS,25,50,75,100 MG/ML.
30 % COAL REFUSE SLURRY
SULFATE (GM./LITER)
0 MG./ML.-CONTROL
EACH 25 MG./ML.
EACH 50 MG./ML
EACH 75 MG./ML.
EACH 100 MG./ML.
0
1
2
3
4
5
6
7
8
9
10
11
12
13
DAYS X 2 (26 TOTAL)

Figure 27

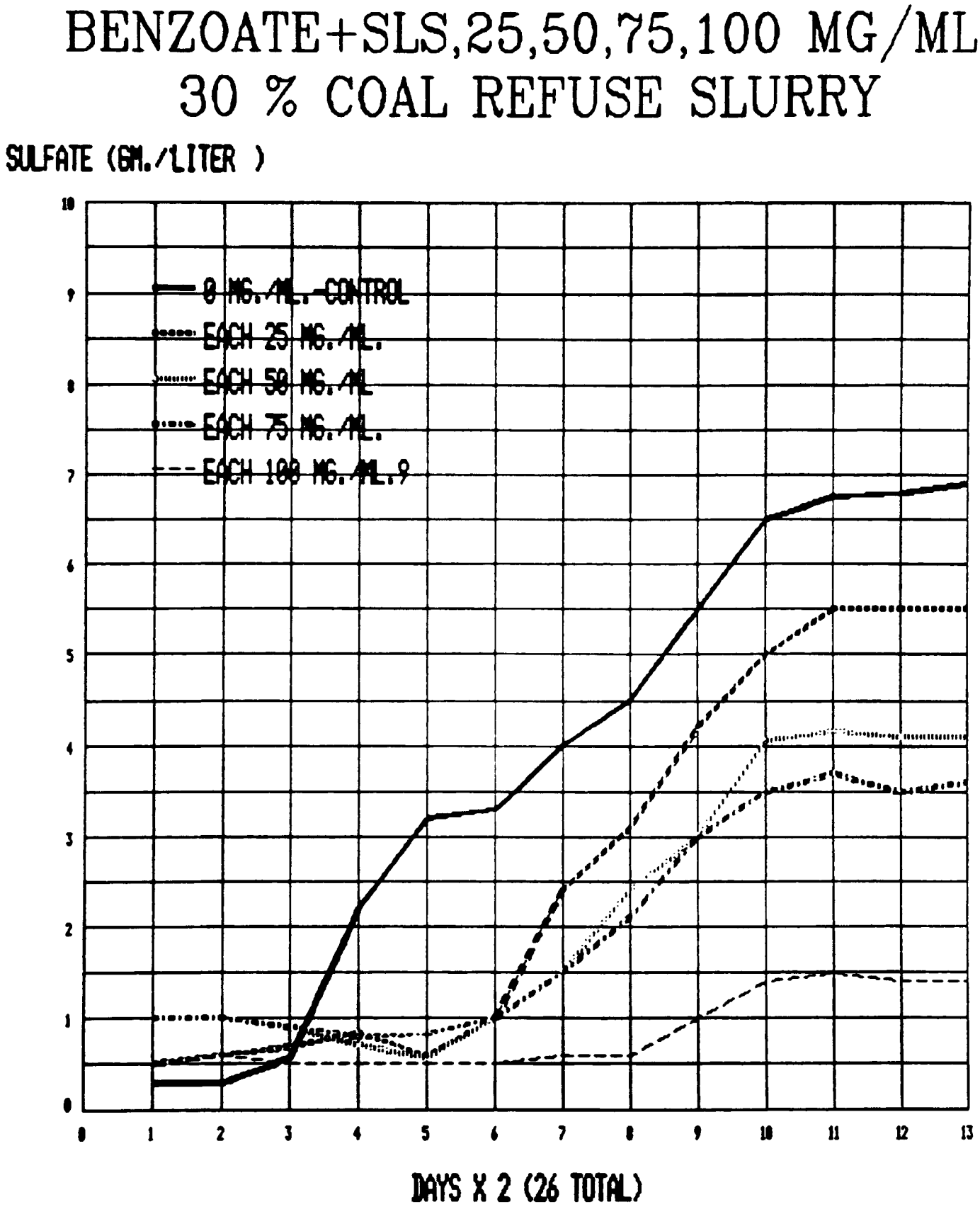
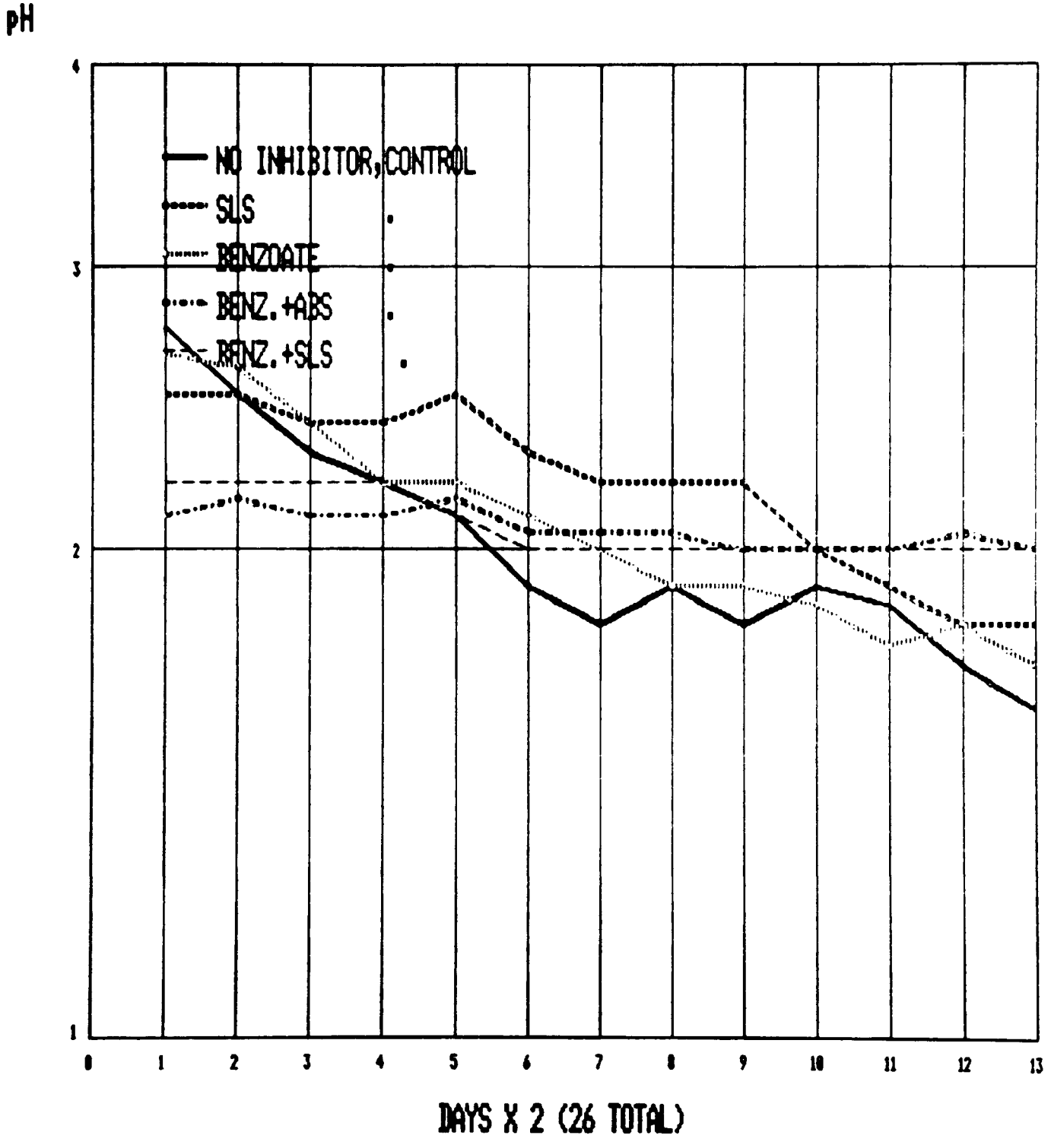

BENZOATE+SLS,25,50,75,100 MG/ML.
30 % COAL REFUSE SLURRY
SULFATE (GM./LITER)
0 MG./ML.-CONTROL
EACH 25 MG./ML.
EACH 50 MG./ML
EACH 75 MG./ML.
EACH 100 MG./ML.
0
1
2
3
4
5
6
7
8
9
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
DAYS X 2 (26 TOTAL)

Figure 28

INHIBITORS, 100 MG./ML., 1% INOCULUM 30 % COAL REFUSE SLURRY



Inhibition of Pyrite Oxidation in Partially Simulated Field Conditions.

Coal refuse is reject coal because of its high content of sulfur, and other minerals (ash). It is not a homogeneous material and the mineral content varies qualitatively and quantitatively. Experiments on pulverized blended refuse in the laboratory, while providing important data, do not simulate field conditions.

The following experiments make use of coal refuse as taken from the field and subjected to inhibitory chemicals under partially controlled laboratory conditions (temperature, precipitation) in an effort to partially simulate field conditions.

Approximately 750 lb. (341 kg) coal refuse (6 - 8% total S) obtained from Peabody Coal Co. Sunny-Hill Mine, New Lexington, Ohio, was placed in each of two bins constructed of welded 10 mm polyethylene (see Figures 29 and 30) and held at 23 +/- 2 ^{o}C. The bins were equipped with a 0.75 in. (19 mm) polyvinyl chloride valve and drain at one end and were raised 1.5 in. (38 mm) at the opposite end to provide drainage. Each bin was sprinkled with 1 gallon (3.67 L.) distilled water per day from Monday through Friday; calculated to be equivalent to the 0.13 in. per day average rainfall (April, May, June) at the mine location. The bins were not inoculated but relied on the natural microflora present in the refuse. Refuse particle size was as taken from the mine.

All drainage from each bin was collected, recorded and analyzed according to the following scheme: SLS where applicable was analyzed (1) in samples collected after Monday sprinkling. Titratable acidity (31) and bacterial counts (39) after Wednesday sprinkling, total iron by atomic absorption after Friday, total SO_4 based on average of 3 samples (20) collected after Monday, Wednesday, and Friday. Of the 5 gallon water (18.85 L.) added/bin/week, an

average of 14.5 L. was recovered indicating an average evaporation of 3.85 L./week. Bacterial enumeration was by standard three-tube dilution series most probable number determination using media composed of the "9K" salts solution (36) for iron oxidizing autotrophs and in "9K" salts which had elemental sulfur substituted for the ferrous iron energy source for the determination of sulfur oxidizing autotrophs and "9K" salts with glucose plus yeast extract substituted for sulfur for the enumeration of heterotrophs.

Each of the two bins were treated identically for the first four weeks in an attempt to establish a biological equilibrium after transfer of coal refuse from field to laboratory. After four weeks one bin was treated on one day during the week (Tuesday) by sprinkling 20g SLS plus 20 g of sodium benzoate which was dissolved in the one gallon of distilled water sprinkled over the surface on Tuesday. The second bin was held as a control and sprinkled only with distilled water. This treatment was repeated weekly for a total of four treatments. The purpose of adding benzoate was to retard the yeasts, fungi and heterotrophic bacteriathat might either degrade the SLS or metabolically remove autotoxic metabolic byproducts synthesized by the Thiobacillus (43). However, the data which shows that Benzoic acid alone is inhibitory to pyrite oxidation, illustrates that it is directly inhibitory to the iron and sulfur oxidizers. Beginning the eighth week each bin was again treated identically with only distilled water. The amount of SLS plus benzoate was selected on the basis of calculations from preliminary experiments carried out in 125 ml. volumes of refuse slurry in 250 ml. shaken Erlenmeyer flasks on the basis of the concentration of SLS plus benzoate which would inhibit pyrite oxidation in refuse. Twenty grams per 341 Kg is approximately equivalent to 60 parts detergent per million parts refuse by weight in the bins.

Results

Results of the experiment are presented in Figures 30 and 31. During the initial four week equilibration period there was some decrease in total iron, sulfate, titratable acidity, and iron and sulfur oxidizing bacteria, probably indicating that the rate of washout from the refuse exceeded oxidation within the refuse; i.e., equilibration had not been reached. The variability of data is to be expected in such a system because refuse size and composition were heterogeneous with aggregate sizes ranging from about 20 cm to 1 micrometer. Some sedimentation occurred within the bin and water channelization as well as pockets of acid formation are likely.

By the time of the third application of SLS (6th week) both iron and sulfur oxidizers had dissappeared from the drainage. However, a considerable amount of SLS was leaching through the bin and these bacteria would not survive in the drainage in the presence of SLS (14,19). There is the probability that pockets of viable iron and sulfur ozidizers were active in the bins due to incomplete contact between SLS and bacteria, i.e., numbers increased after the SLS flushed out (8-10th week). During the period after treatment began (4th through 10th week) the control bin leached increasing amounts of iron anc sulfate and the bacterial counts remained high, showing a marked comparative reduction of pyrite oxidation and acid formation between control and treated bin. It should be noted that bacterial enumeration by the technique used (i.e., MPN) required two weeks incubation time and the data were not known at the time of repeated SLS-benzoate application. The data indicate that frequency of application could have been reduced.

There is also a marked difference in titratable acidity after the seventh week. A longer response time for difference in titratable acidity to become

apparent is anticipated because any pyritic iron which had been oxidized to th ferric ion, prior to bacterial inhibition, is already committed to acid formation in accordance with equation IV (18).

Summary of Experiments with 750 Pounds Coal Refuse in Bins.

Two bins each containing 750 lbs. coal refuse

No inoculum, No pulverization, 23 °C.+/- 2 °C., 14 weeks

Each bin sprinkled with 1 gallon distilled water (3.8 L.),

5 days per week

Bin # 1. Control, No Treatment

Bin # 2. Sodium lauryl sulfate (SLS) plus sodium benzoate added: 4 weekly applications of 1 gallon (3.8 L.) of solution which contained 20 gm. SLS plus 20 gm. benzoic acid (as sodium salt) per gallon.

Figure 29

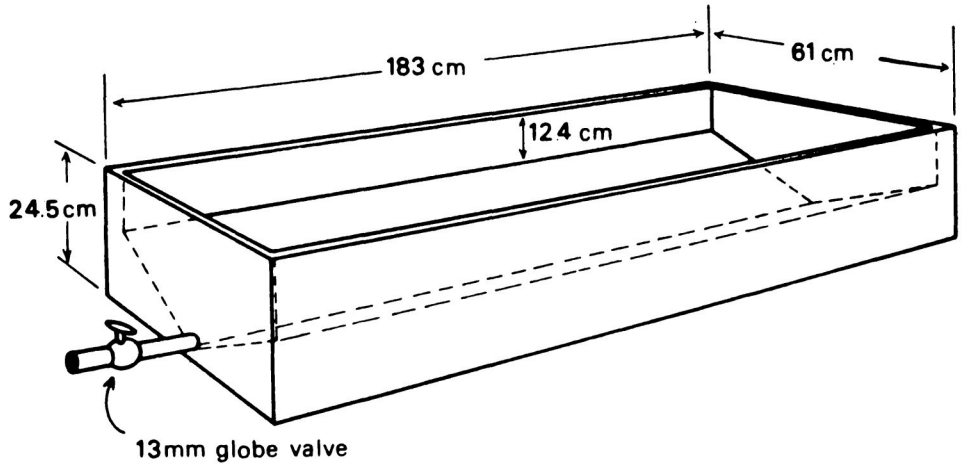


Figure 30

Figure 31

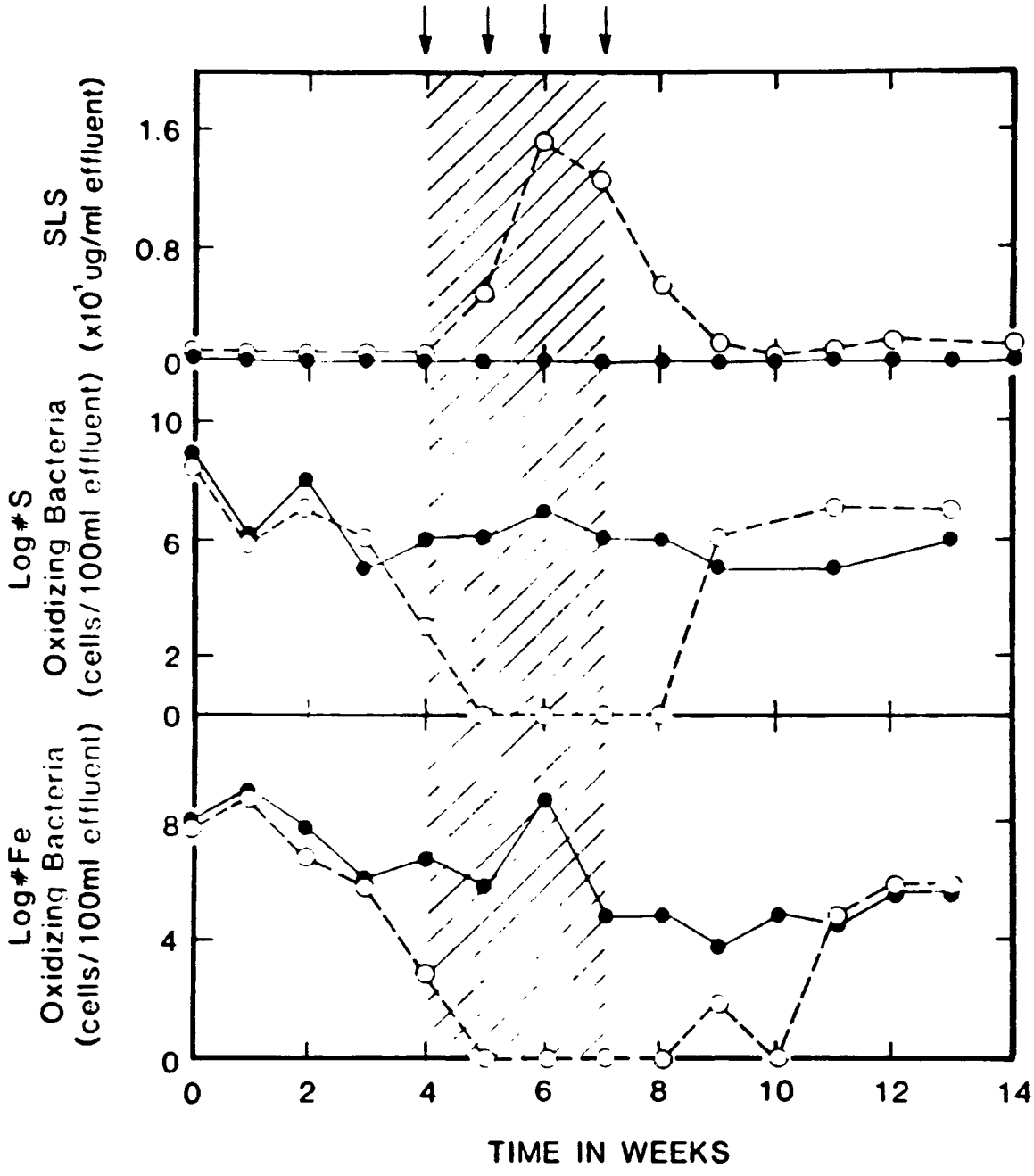
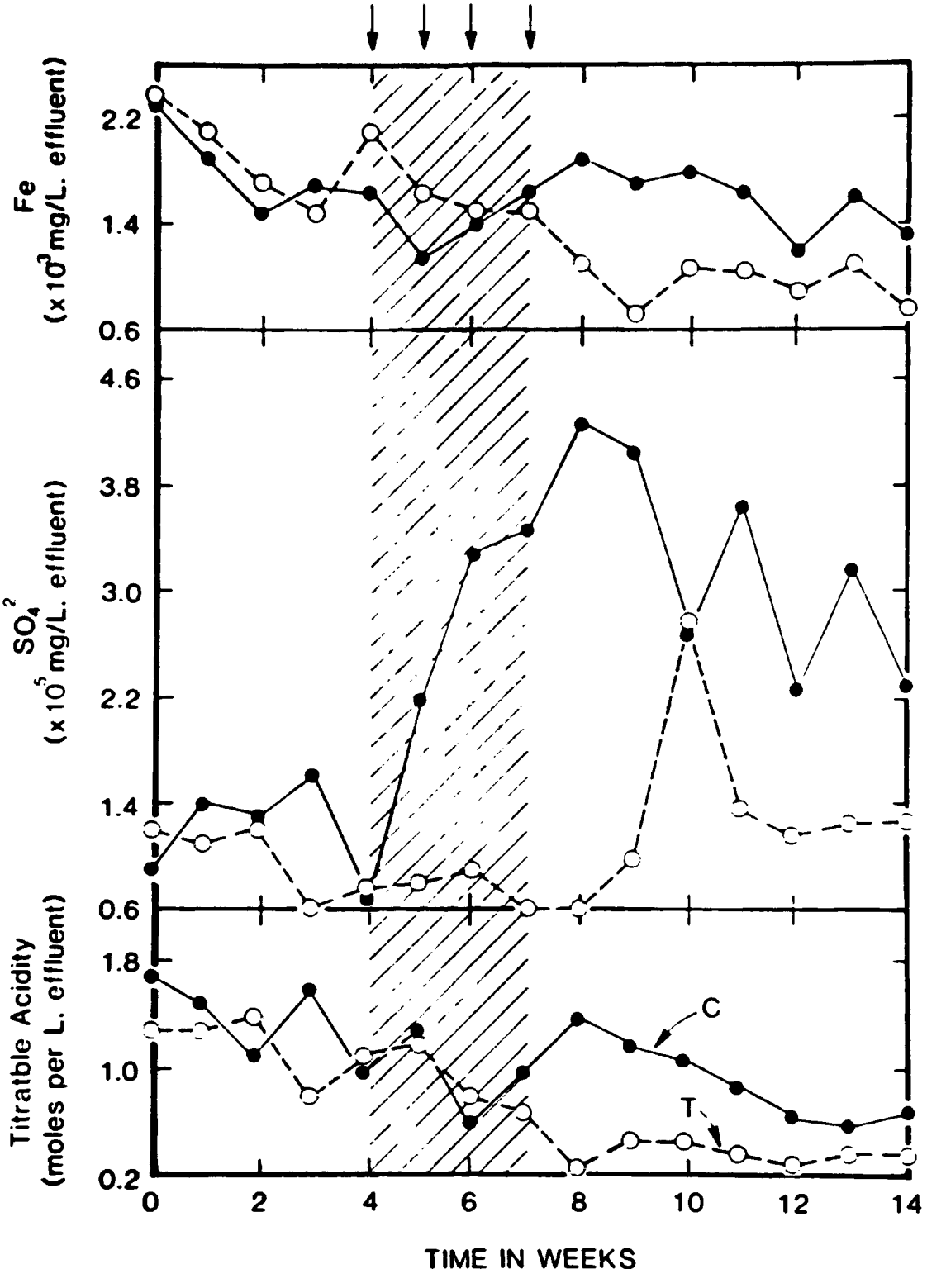

SLS
(x10³ug/ml effluent)
1.6
0.8
0
Log#S
Oxidizing Bacteria
(cells/100ml effluent)
10
6
2
0
Log#Fe
Oxidizing Bacteria
(cells/100ml effluent)
8
4
0
0
2
4
6
8
10
12
14
TIME IN WEEKS

Figure 32


Fe
(x 10³ mg/L. effluent)
2.2
1.4
0.6
SO₄²
(x 10⁵ mg/L. effluent)
4.6
3.8
3.0
2.2
1.4
0.6
Titratble Acidity
(moles per L. effluent)
1.8
1.0
0.2
C
T
0
2
4
6
8
10
12
14
TIME IN WEEKS

Inhibition of Pyrite Oxidation in 750 pound Bins of Coal Refuse in Presence and Absence of Lime.

Lime is frequently used to neutralize acid spoil and coal refuse in the field during reclamation.

Four bins, each containing 750 pounds of refuse obtained at the Peabody Sunny-Hill mine were allowed to equillibrate for 4 weeks at ambient temperature. Each bin was sprinkled on 5 successive days each week (Mon., Tues., Wed. Thurs. Fri.) with 1 gallon (3.8 L.) distilled water.

Beginning with the fourth week, bins #2 and #4 each had 200 grams of lime spread evenly over the surface and mixed into the top 4" to 6" layer of refuse with the aid of a wooden spatula. This treatment was repeated once each week for a total of 4 treatments (800 gm. lime total per bin).

In addition, a 1 gallon solution (3.8 L.) containing 100 milligrams SLS plus 100 milligrams benzoic acid (as sodium benzoate) per liter was sprinkled over bins #3 and #4, once each week from the 4th to 7th week (4 treatments) in place of 1 gallon water. Bin #1 received only distilled water and served as an untreated control.

Results

Lime treatment reduced sulfate in the effluent compared to the control (Figure 33) during weeks 4 through 9. This was not attributed to inhibition of pyrite oxidation because neither the iron content (Figure 35) nor the microbial content (Figures 38, 39 and 40) of the effluent was significantly diminished during that time. pH and titratable acidity did decrease, showing that neutralization took place.

The effect of SLS plus benzoic acid on sulfate reduction was significant (Figure 33) and this was paralleled by reduction in iron (Figure 34) as well

as titratable acidity (Figure 35), pH (Figure 36) and decreased bacterial counts (Figures 38, 39 and 40). The sudden increases in measured parameters followed by rapid decreases (e.g. 8th to 10th week) coincided with leaching of SLS through the refuse (Figure 37) and probably were due to detergent affecting a sudden washout of soluble substances and small particles (including some resistant microorganisms). Reduction in pyrite oxidation appeared to be immediate upon addition of SLS plus benzoate (week 4 to 5) and increased through week 8 to 9. This represents an approximate 1 to 2 week lag in maximum effect beyond the cessation of treatment (week 7).

The number of iron and sulfur oxidizers recovered significantly in 3 to 5 weeks after treatment with SLS and benzoic acid was terminated (week 7). This was also the case for heterotrophic microorganisms.

Figure 41 presents the weekly effluent recovery data from each bin. In comparison to the 18.9 Liters added each week, approximately 20% to 35% moisture was lost each week to evaporation. Humidity was not controlled and the greatest evaporation appeared to coincide with a rise in temperature (Figure 42).

Summary of Additional Experiment on 750 pound Bins of Coal Refuse.

4 Container bins (See Figure 29) each with 750 pounds of coal refuse
No inoculum, 22 C.+/- 2 C., 13 weeks

Each bin sprinkled with 1 gallon distilled water (3.8 L.) 5 days per week (Mon.,Tues.,Wed.,Thurs.,Fri.)

Bin # 1. Control, no treatment

Bin # 2. Lime added: 4 weekly applications of 200 grams each (total 800 gm. = 1.76 pounds)

Bin # 3. Sodium lauryl sulfate plus sodium benzoate added: 4 weekly applications of 1 gallon (3.8 L.) of solution which contained 100 mg. SLS per L. plus 100 mg. benzoic acid per L. (as sodium salt).

Bin # 4. Lime added as per bin # 2 plus SLS and benzoic acid added as per Bin # 3.

Figure 33. Average Sulfate concentration (grams per 100 ml.) in cumulative weekly effluent from each bin over the 13 week period.

Figure 34. Average total dissolved iron concentration (1000 mg./L.) in cumulative weekly effluent from each bin.

Figure 35. Titratable acidity as moles acid per Liter of effluent from each bin.

Figure 36. Average pH of weekly effluent

Figure 37. Average sodium lauryl sulfate (SLS) concentration (Milligrams SLS per Liter) x100 from each tank.

Figure 33

SULFATE IN WEEKLY EFFLUENT FROM 750 LB.COAL REFUSE SAMPLES

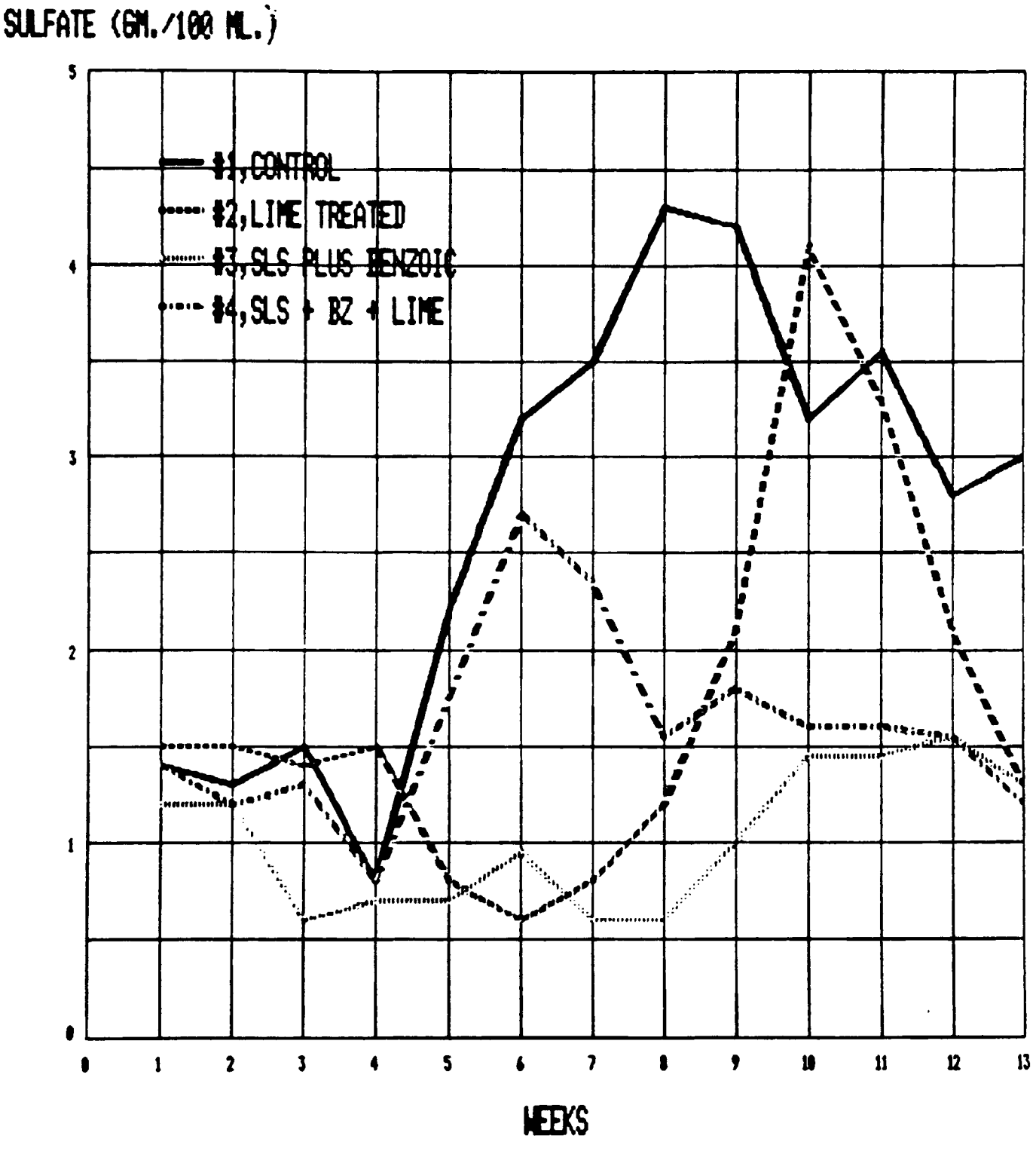


Figure 34

TOTAL IRON IN WEEKLY EFFLUENT FROM 750 LB.COAL REFUSE SAMPLES

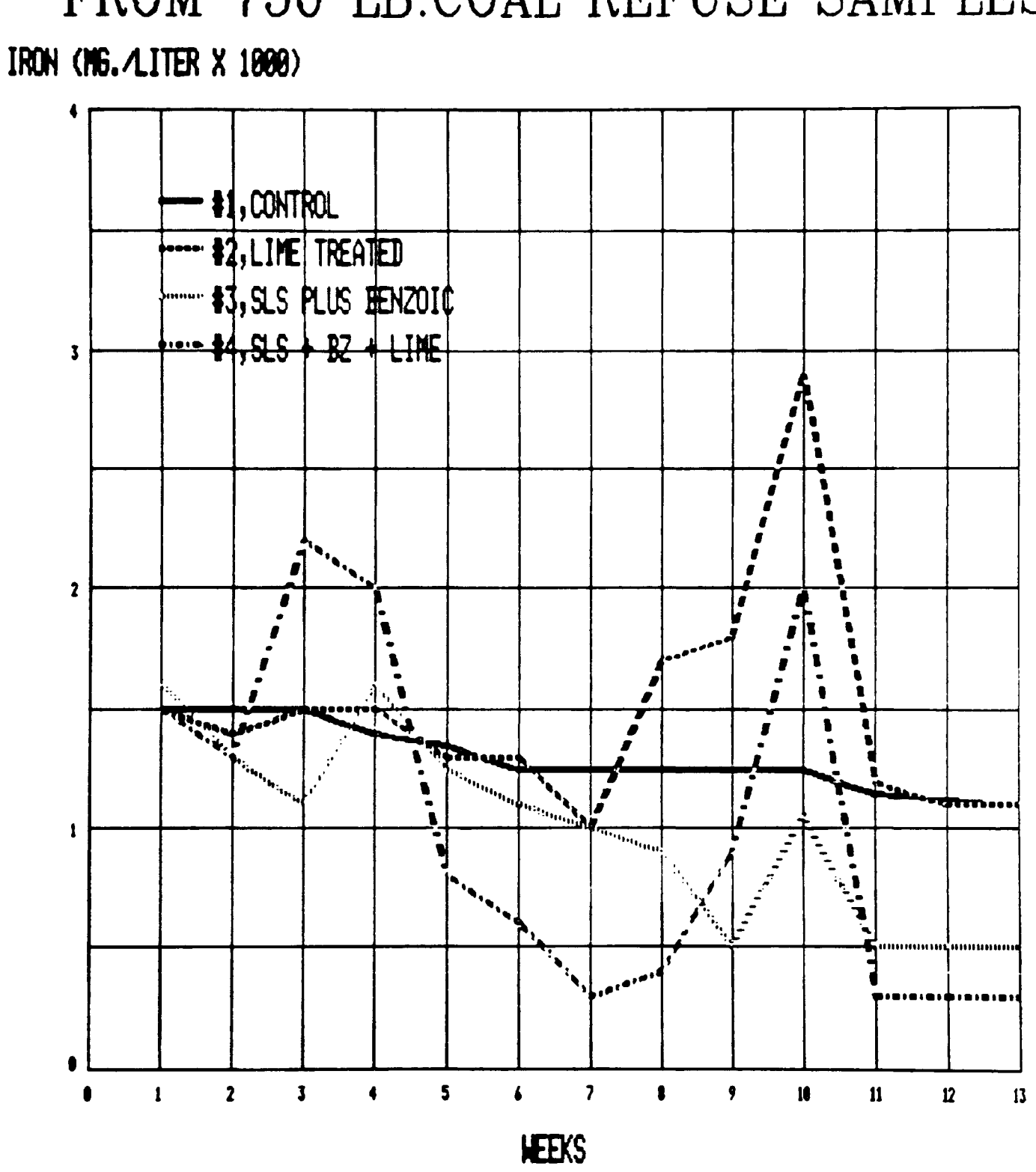


Figure 35

TITRATABLE ACIDITY (M/L) IN EFFLUENT FROM 750 LB.COAL REFUSE SAMPLES

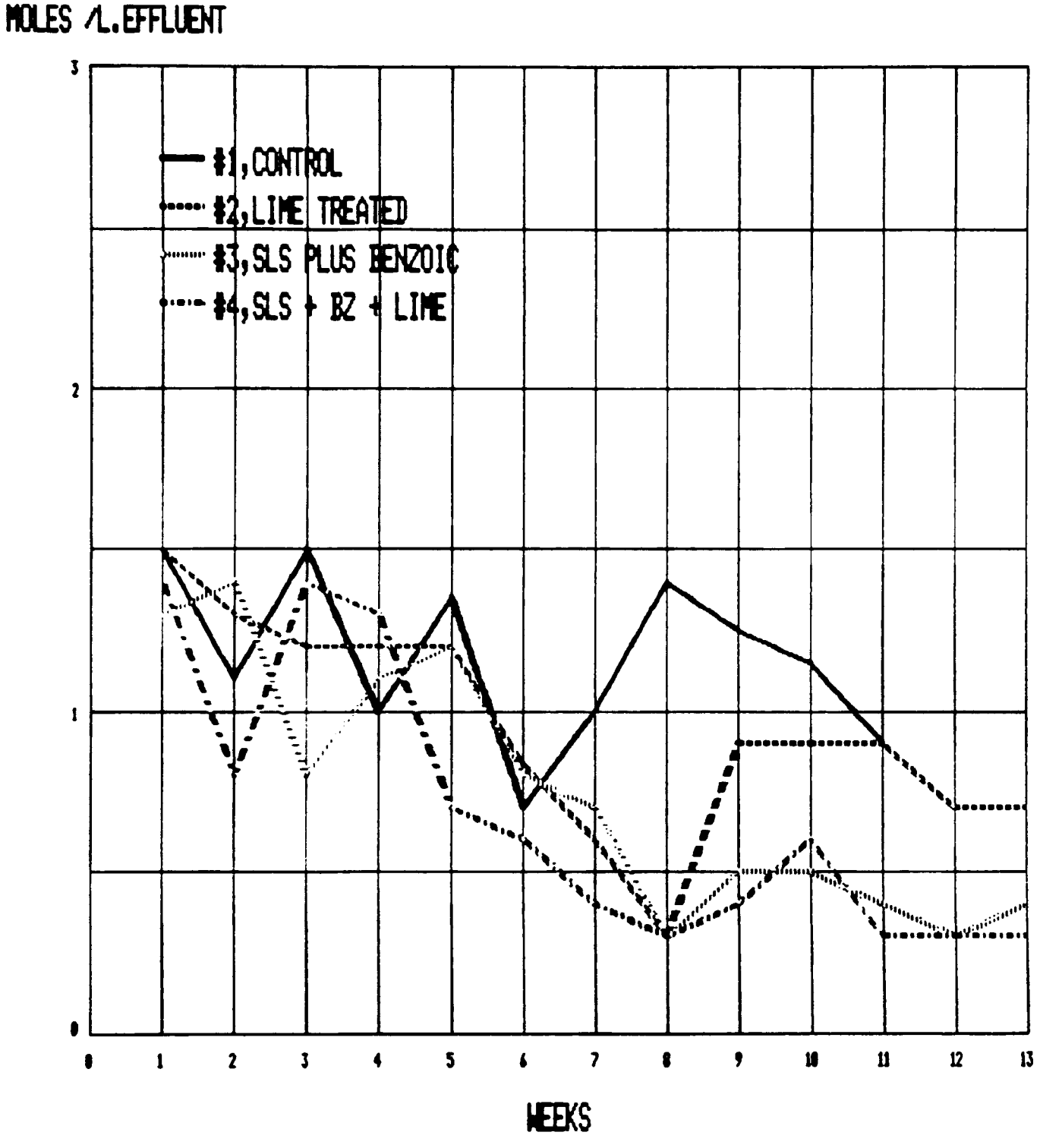


Figure 36

pH OF EFFLUENT FROM 750 LB.COAL REFUSE SAMPLES

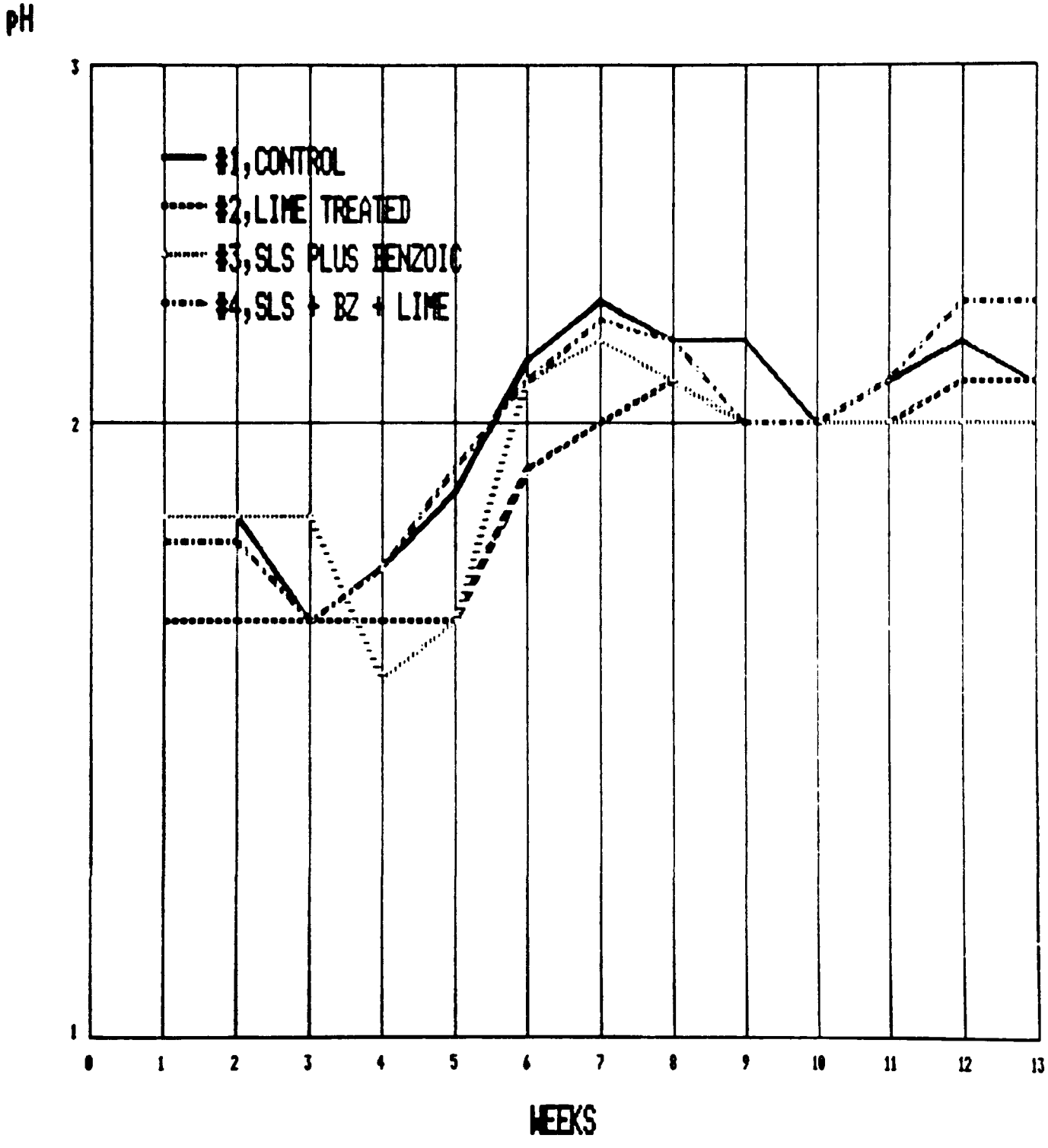


Figure 37

SLS,MILLIGRAM/LITER OF EFFLUENT(X 100) FROM 750 LB.COAL REFUSE SAMPLES

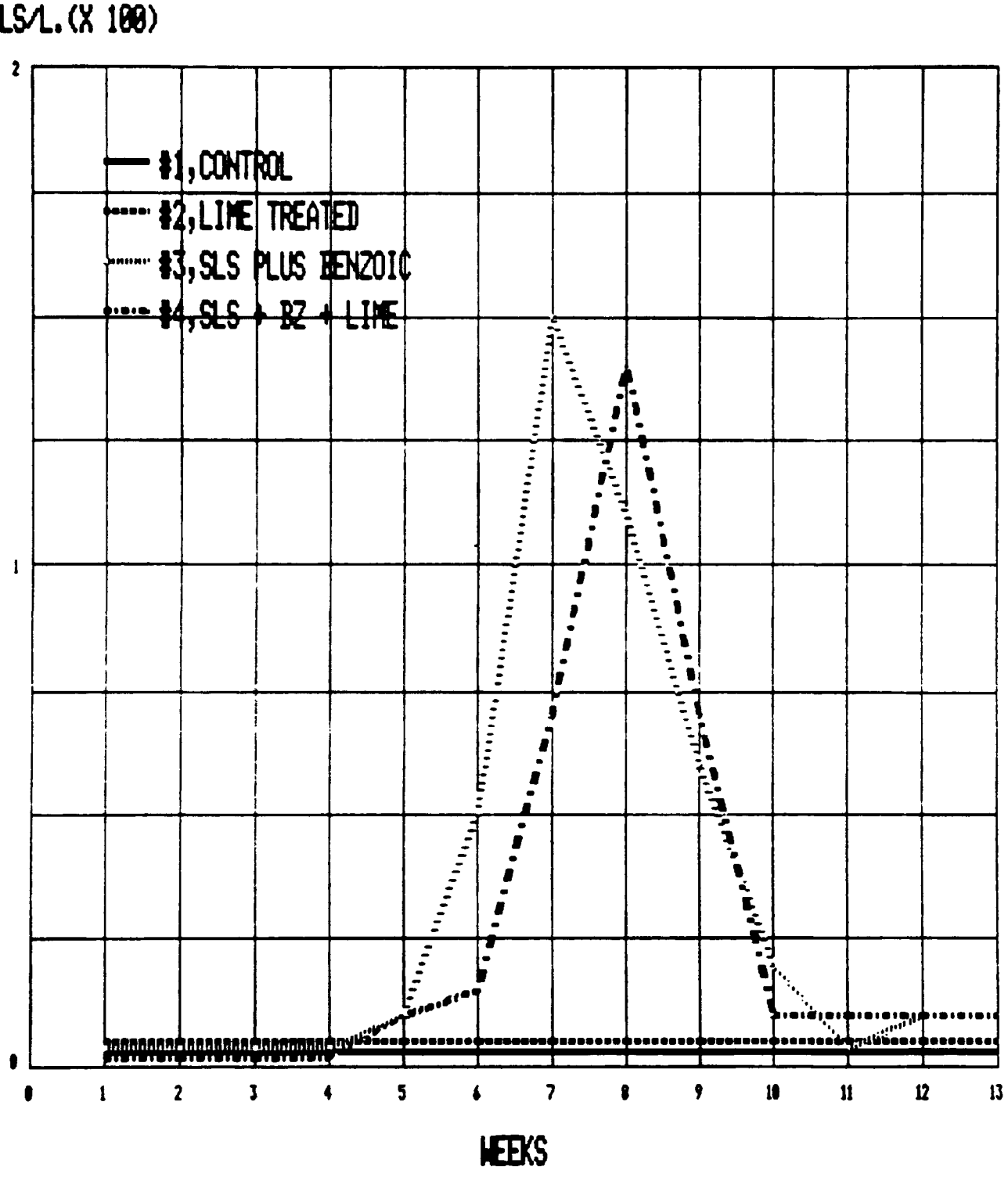


Figure 38

IRON OXIDIZING BACTERIA IN EFFLUENT FROM 750 LB.COAL REFUSE SAMPLES

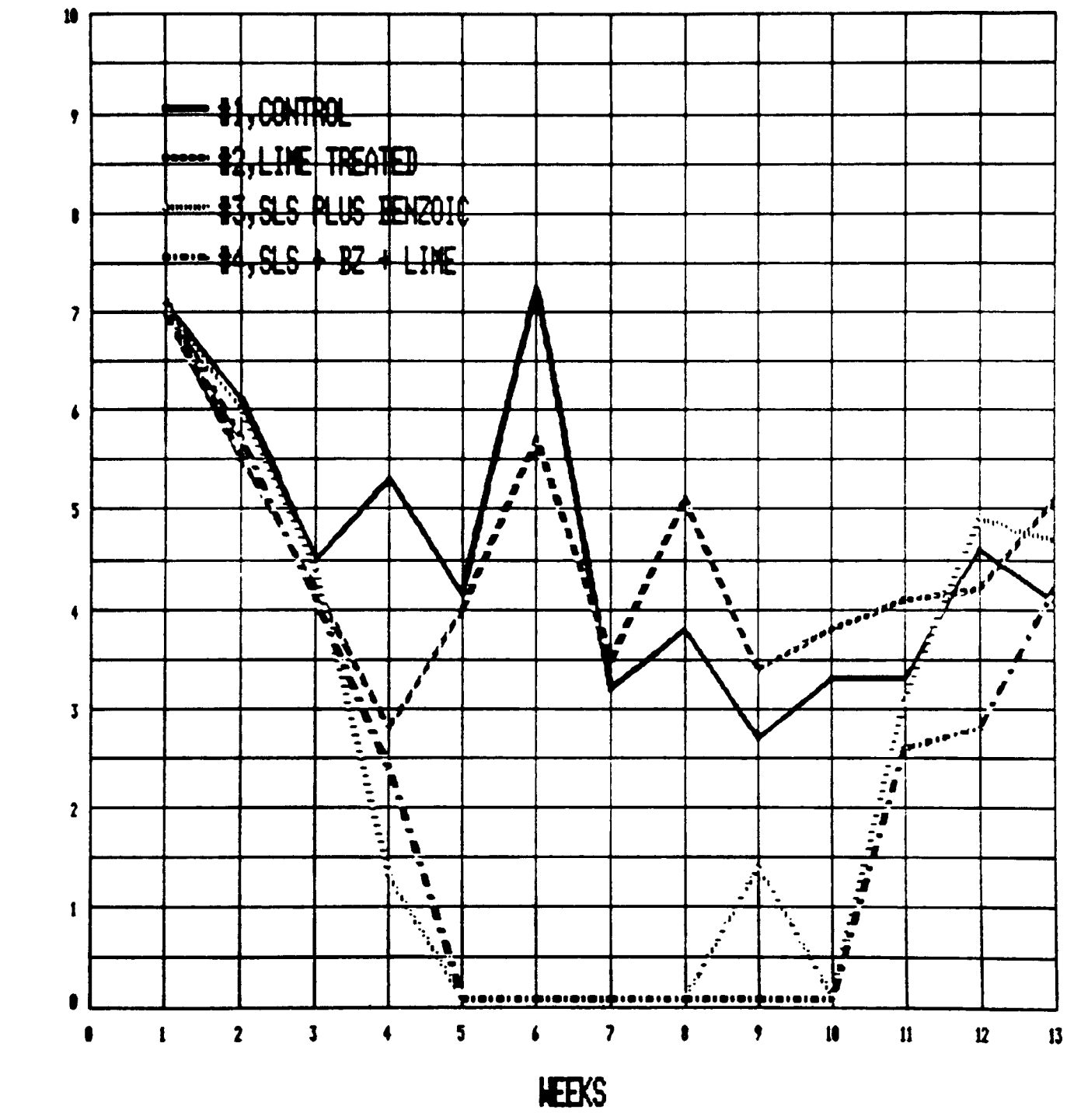


Figure 39

SULFUR OXIDIZING BACTERIA IN EFFLUENT FROM 750 LB.COAL REFUSE SAMPLES

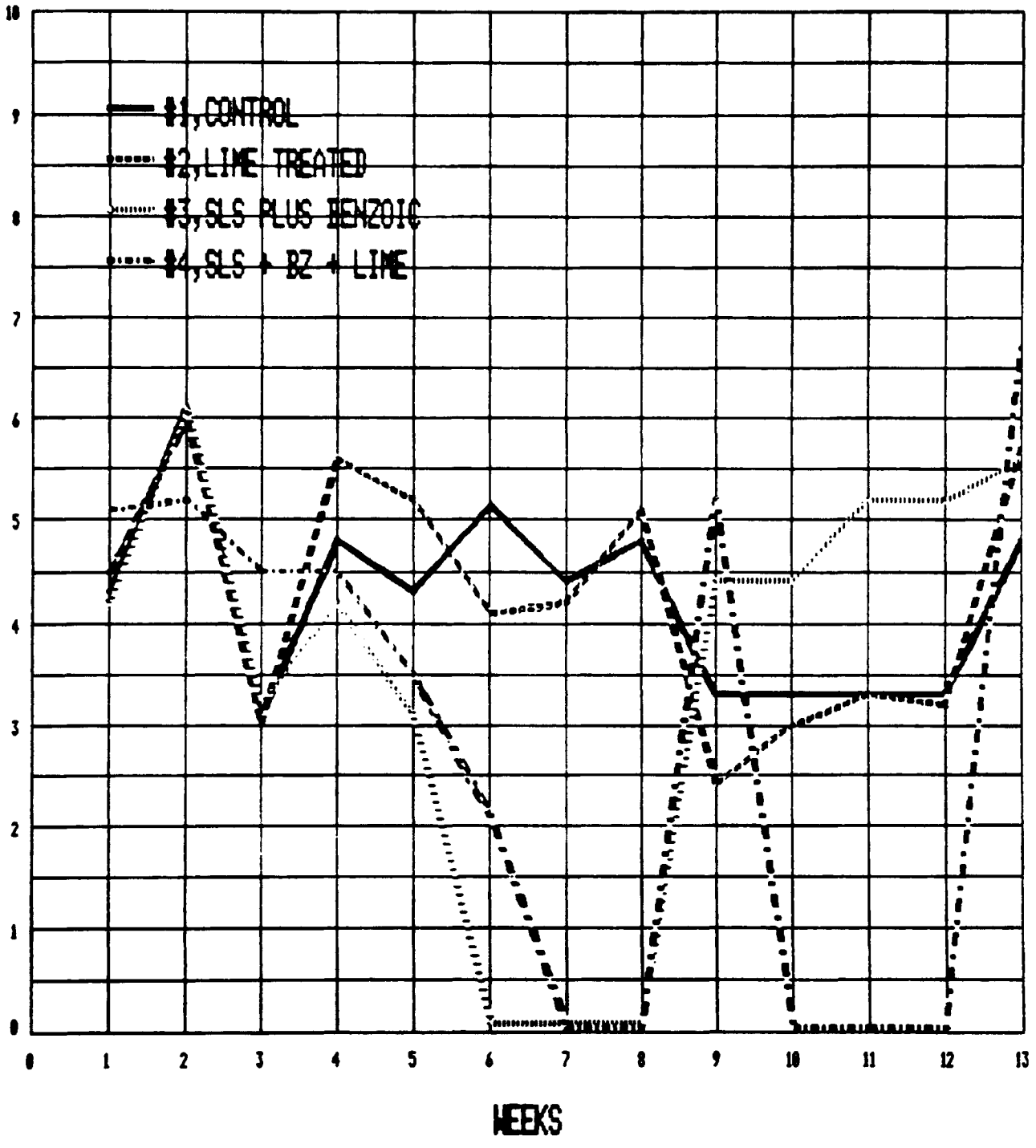


Figure 40

HETEROTROPHIC MICROBES IN EFFLUENT FROM 750 LB.COAL REFUSE SAMPLES

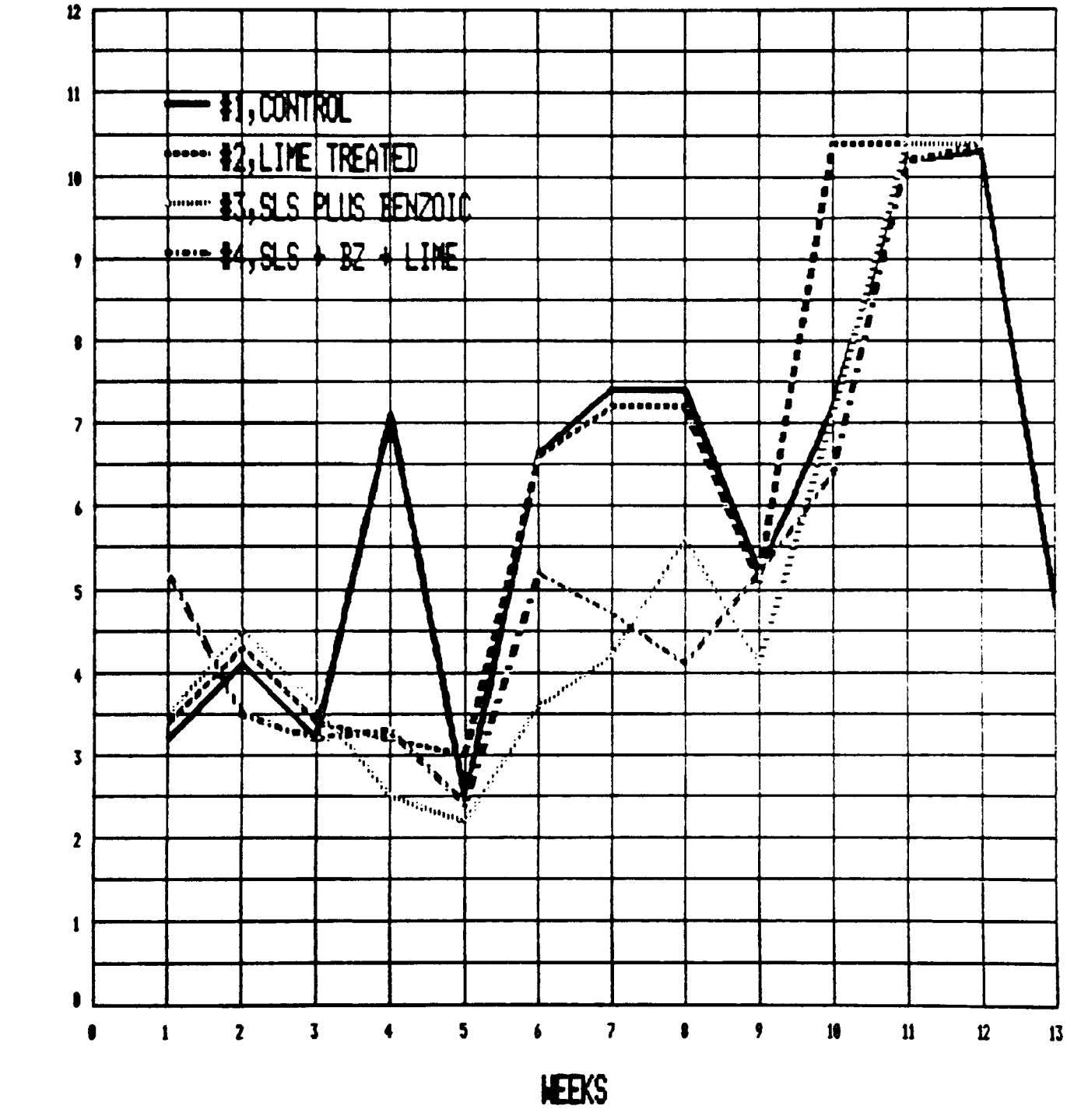


Figure 41

WEEKLY EFFLUENT RECOVERY FROM 750 LB.COAL REFUSE SAMPLES

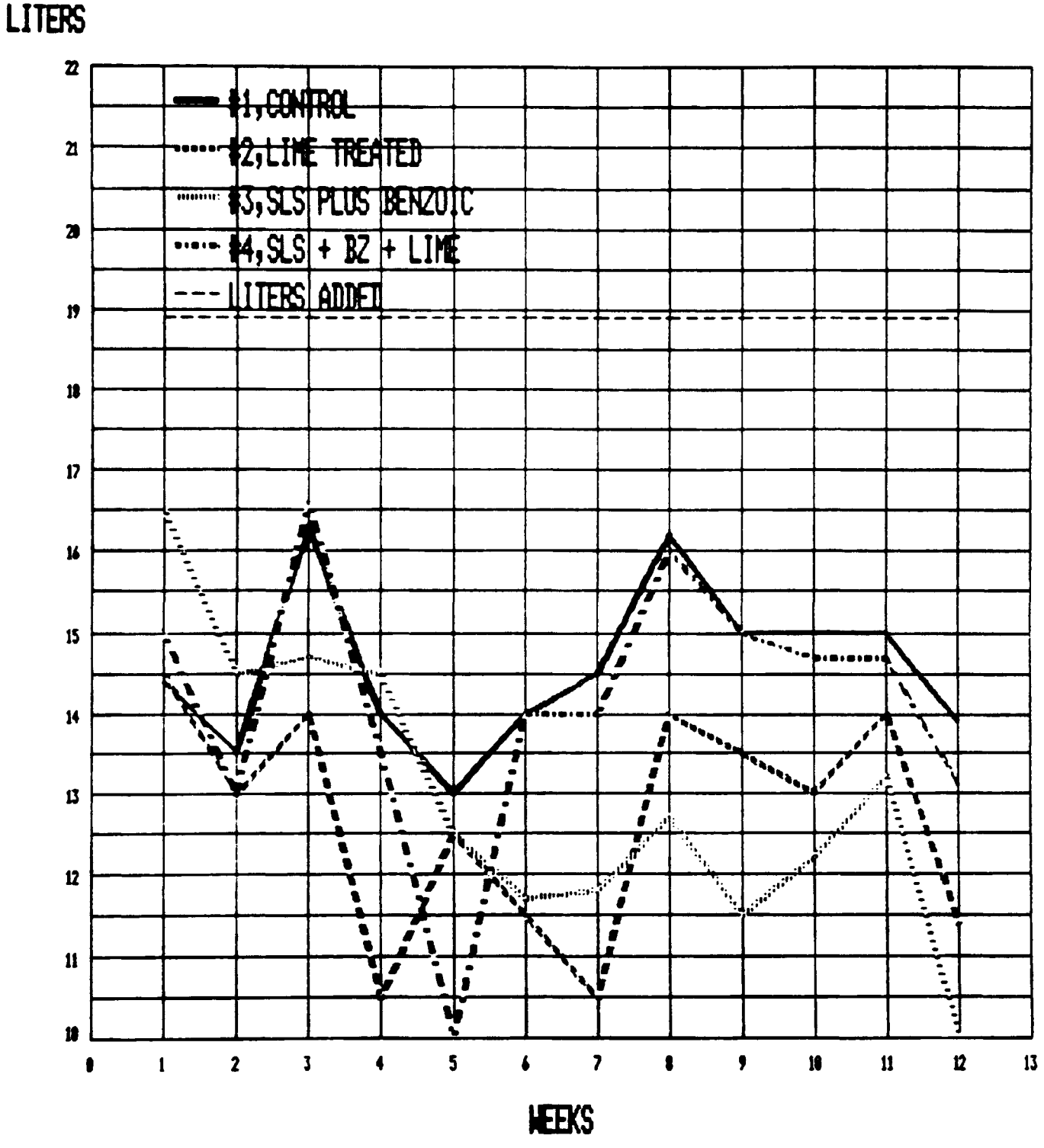
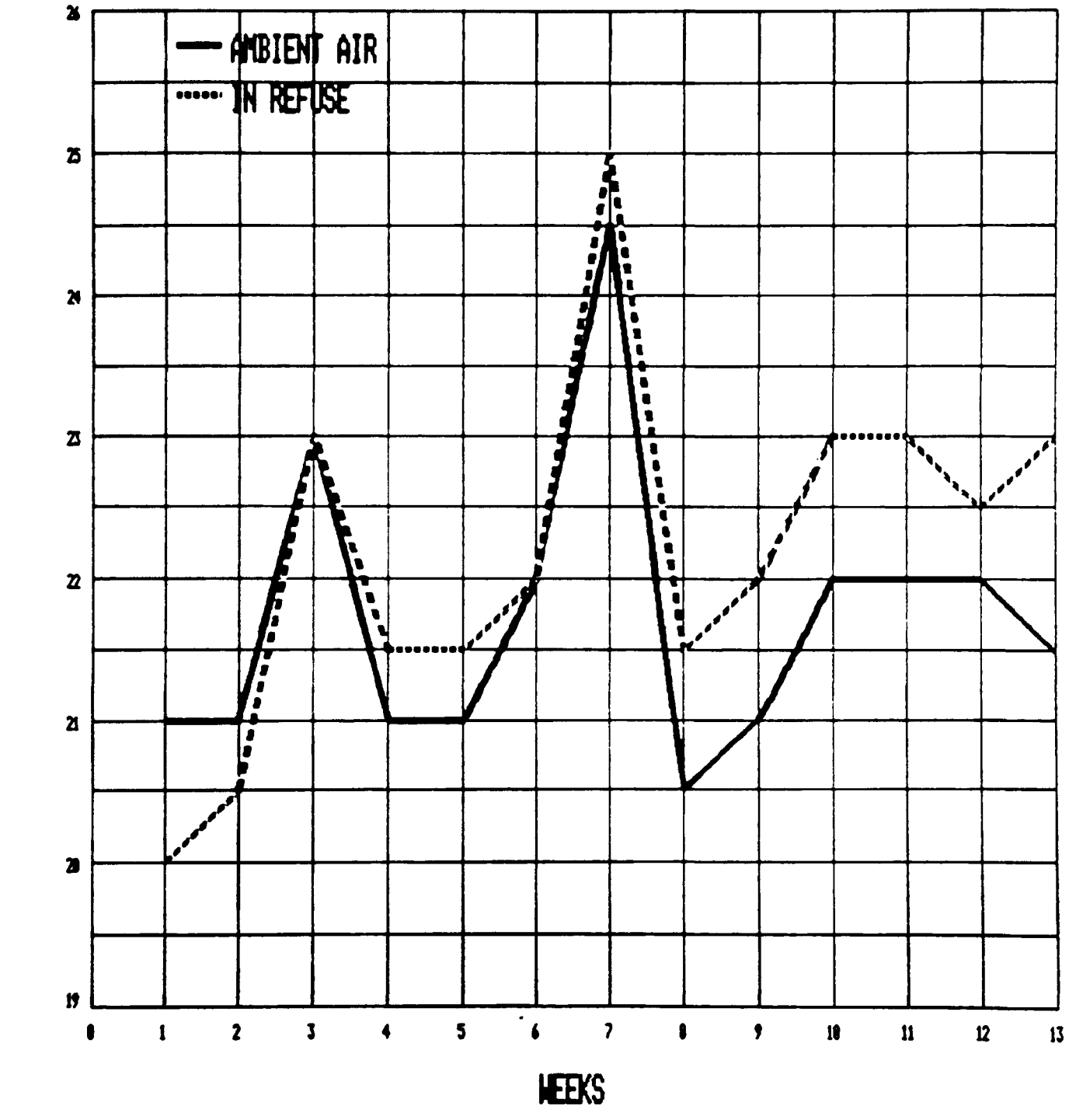


Figure 42

AVG.WEEKLY TEMPERATURE,DEGREES C AMBIENT AND IN REFUSE CONTAINER



SUMMARY

The data presented in this report demonstrate that it is possible to inhibit pyrite oxidizing bacteria in high sulfur coal refuse with a concurrent reduction in acid drainage formed in the refuse.

The most effective inhibitors studied are combinations of sodium lauryl sulfate (SLS) plus sodium benzoate (Bz), both of which are relatively non-toxic to higher organisms. Bz is approved as a human food additive and SLS is a commonly used anionic detergent that is readily biodegradeable. Both are relatively inexpensive substances that are commercially available. SLS and Bz were effective alone but more effective in combination. 100 milligrams per liter was an effective concentration in either 30% refuse slurries or in actual coal refuse. The inhibitory response of SLS and Bz was immediate but both the organisms and pyrite oxidation re-appeared within 2 to 5 weeks after treatment was terminated and the SLS leached out of the refuse. SLS and Bz were effective in the presence of lime, a chemical frequently used to naturalize acid spoils and acid drainage during reclamation.

Alkyl benzene sulfonate (ABS) is also an effective inhibitor although it is required in slightly higher concentrations than SLS to acheive equal reduction of acid formation. Some organic acids are effective inhibitors (acetic, hexanoic, propionic, pyruvic) when present in considerably higher concentrations compared to SLS, ABS or Bz. The lignin sulfonate formulations examined were ineffective.

Concentrations of detergent below the effective inhibitory amount actually stimulated the rate of pyrite oxidation in refuse compared to the control rate. Caution should be exercised when applying inhibitors in the field to insure that effective doses are used.

Although the data presented are variable, any expanded field trials are likely to show even greater variability due to uncontrolled environmental influences such as rainfall, humidity, temperature and variability of refuse with respect to depth, composition and particle size. The qualitative variability in composition of coal refuse and coal spoil must be emphasized. e.g. Its content of sulfur, pyrite, other metal sulfides, silicate, metal oxides, etc., varies considerably from location to location within short distances. These experiments represent a scale-up from highly controlled laboratory experiments and demonstrate that pyrite oxidizing bacteria can be inhibited with a concomitant reduction in formation of acid drainage in the field. The net effect will be reduced environmental damage and reduced economic loss caused by acid drainage. It will also provide additional time to re-vegetate during reclamation before acid retards the new growth. Kleinmann et al. have demonstrated acid drainage reduction by application of detergent embedded within a rubber matrix to promote slow controlled release of the detergent (26). Detergents may also be amenable to use in abandoned deep mines if added to water prior to its flow through the mine where it comes into contact with bacteria and pyrite.

LITERATURE CITED

1. Abbott, D.C. 1962. The colorimetric determination of anionic surface active materials in water. The Analyst 87: 286-290.

2. Acid Mine Drainage in Appalachia. 1969. Appendix C: The incidence and formation of mine drainage pollution in Appalachia. 253 pp. U.S. Corps of Engineers and U.S. Dept. of Interior.

3. Beck, J. 1960. A ferrous-ion-oxidizing bacterium. I. Isolation and some general physiological characteristics. J. Bacteriology 79:502-509.

4. Belly, R.T. and T.D. Brock. 1974. Ecology of iron-oxidizing bacteria in pyritic materials associated with coal. J. Bacteriology 117:726-732.

5. Blaylock, B.A. and A. Nason. 1963. Electron transport systems of the chemoautotroph Ferrobacillus ferrooxidans. I. Cytochrome c-containing iron oxidase. J. Biol. Chem. 238:3453-3462.

6. Bodo, C.A. and D.G. Lundgren. 1972. Oxidation of iron by cell-free envelopes of Thiobacillus ferrooxidans. Abstr. A.S.M. 72:174.

7. Borichewski, R.B. 1967. Keto acids as growth-limiting factors in autotrophic growth of Thiobacillus thiooxidans. J. Bacteriology 93:597-599.

8. Carpenter, E.V. and L.K. Henderson. 1933. Acid mine drainage from bituminous coal mines. Res. Bull. No. 10. Engineering Experiment Station, Univ. of West Virginia.

9. Colmer, A.R. and M.E. Hinkle. 1947. The role of microorganisms in acid mine drainage: A preliminary report. Science 106:253-256.

10. Colmer, A.R., K.L. Temple and M.E. Hinkle. 1949. An iron oxidizing bacterium from the acid drainage of some bituminous coal mines. J. Bacteriology 59:317-328.

11. Cook, W.B. 1966. The occurrence of fungi in acid mine drainage. Proc. Indust. Waste Conf. 21:258-274.

12. Dewey, D.L. and J. Beecher. 1966. The internal hydrogen ion concentration of Thiobacillus thiooxidans and survival after irradiation. Radiation Research 28:289-295.

13. Din, G.A., I. Suzuki and H. Lees. 1967. Ferrous iron oxidation by Ferrobacillus ferrooxidans. Purification and properties of Fe++-cytochrome C reductase. Can. J. Biochem. 45:1523-1546.

14. Dugan, P.R. and D.G. Lundgren. 1964. Acid production by Ferrobacillus ferrooxidans and its relation to water pollution. Develop. in Industrial Microbiology. 5:250-257.

15. Dugan, P.R. and C.I. Randles. 1968. The microbial flora of acid mine water and its relationship to formation and removal of acid. Rept. Ohio State Univ. Water Resources Center, Columbus, Ohio. 123 pp.

16. Dugan, P.R., C.B. Macmillan and R.M. Pfister. 1970. Aerobic heterotrophic bacteria indigenous to pH 2.8 acid mine water: Microscopic examination of acid streamers. J. Bacteriology 101:973-981.

17. Dugan, P.R., C.B. Macmillan and R.M. Pfister. 1970. Aerobic heterotrophic bacteria indigenous to pH 2.8 mine water: Predominant slime-producing bacteria in acid streamers. J. Bacteriology 101:982-988.

18. Dugan, P.R. 1972. Biochemistry of acid mine drainage. In: Biochemical Ecology of Water Pollution. pp. 123-137. Plenum Publ. Co., N.Y., N>Y>

19. Dugan, P.R. 1975. Bacterial ecology of strip mine areas and its relationship to the production of acidic mine drainage. Ohio J. Sci. 75:266-279.

20. Dugan, P.R., and W.A. Apel. 1977. Microbiological desulfurization of coal. IN Proc. Internat. Symp. Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena. Academic Press, New York. pp. 223-250.

21. Dugan, P.R., U.S. Patent No. 4,456,688 (1984).

22. Dugan, P.R. and W.A. Apel. 1983. Bacteria and Acidic Drainage from Coal Refuse: Inhibition by Sodium Laurly Sulfate and Sodium Benzoate. Applied and Environmental Microbiol. 46:279-282.

23. Duncan, D.W., J. Landesman and C.C. Walden. 1968. Role of Thiobacillus ferrooxidans in the oxidation of sulfide minerals. Can. J. Microbiol. 13:397-403.

24. Good, D.M., V.T. Ricca and K.S. Shumate. 1970. The relation of refuse pile hydrology to acid production. In 3rd Symp. on Coal Mine Drainage Research, Mellon Inst., Pittsburgh, Pa. pp. 145-151.

25. Howard, A. and D. Lundgren. 1970. Inorganic pyrophosphatase from Ferrobacillus ferrooxidans (Thiobacillus ferrooxidans). Can. J. Biochem. 48:1302.

26. Kleinmann, R.L.P., D.A. Crerarm and R.P. Pacelli. 1981. Biogeochemistry of acid mine drainage and a method to control acid formation. Mining Engineering, March, 1981.

27. Land Reborn. 1974. Report by: Board on Unreclaimed Strip Mined Lands and Ohio Department of Natural Resources. Columbus, Ohio 91 pp.

28. Leathan, W.W., S.A. Braley and L.D. McIntyre. 1953. The role of bacteria in the formation of acid from certain sulfuritic constituents associated with bituminous coal. I. Thiobacillus thiooxidans. Appl. Microbiol. 1:61-64. II. Ferrous iron oxidizing bacteria. App. Microbiol. 1:65-68.

29. Lorenz, W.C. and R.W. Stephan. 1967. Factors that affect the formation of coal mine drainage pollution in Appalachia. Rept. Bureau of Mines, U.S. Dept. Interior. Pittsburgh, Pa.

30. Lundgren, D.G., J.R. Vestal and F.R. Tabita. 1974. The iron oxidizing bacteria. In Microbial iron metabolism. (Ed.) J.B. Neilands. Ch. 18. Academic Press, N.Y., N.Y.

31. Payne, D.A., and T.E. Yeates. 1970. The effects of magnesium on acidity determinations of acid mine drainage. In Proc. Third Symp. Coal Mine Drainage Res. Mellon Institute, Pittsburgh, Pa. pp. 200-226.

32. Shumate, K.S. E.E. Smith, P.R. Dugan, R.A. Brandt and C.I. Randles. 1971. Acid Mine Drainage Formation and Abatement. Report U.S. Environmental Protection Agency, Water Pollution Control Series DAST-42, 14010 FPR. Washington, D.C.

33. Silver, M. and D.G. Lundgren. 1968a. Sulfur oxidizing enzyme of Ferroobacillus ferrooxidans (Thiobacillus ferrooxidans). Can. J. Biochem. 46:457-461.

34. Silver, M. and D.G. Lundgren. 1968b. The thiosulfate oxidizing enzyme of Ferroobacillus ferrooxidans (Thiobacillus ferrooxidans). Can. J. Biochem. 46:1215-1220.

35. Silverman, M.P. and H.L. Ehrlich. 1964. Microbial formation and degradation of minerals. Adv. in Appl. Microbiol. 4:153-206.

36. Silverman, M.P. 1967. Mechanism of bacterial pyrite oxidation. J. Bacteriology 94:1046-1051.

37. Singer, P.C. and W. Stumm. 1970. Acidic mine drainage: The rate limiting step. Science 167:1121-1124

38. Sokolova, G.A. and G.I. Karavaiko. 1968. Physiology and Geochemical Activity of Thiobacilli. Trans. from Russian. Israel Program for Scientific Transl., Jerusalem. Through U.S. Dept. Commerce, Springfield, Va. 293 pp.

39. Standard Methods for Examination of Water and Wastewater, 14th Edition. 1976. Publ. American Public Health Assoc., Washington, D.C.

40. Truax-Traer Coal Co. 1971. Control of mine drainage from coal mine mineral wastes. Phase I. Hydrology and Related Experiments. Report. U.S. Environmental Protection Agency, Water Pollution Control Research Series 14010DDH. 148pp.

41. Tuttle, J.H., C.I. Randles and P.R. Dugan. 1968. Activity of microorganisms in acid mine water. I. Influence of acid water on aerobic heterotrophs of a normal stream. J. Bacteriology 95:1495-1503.

42. Tuttle, J.H., and P.R. Dugan. 1976. Inhibition of growth, iron and sulfur oxidation in **Thiobacillus ferrooxidans** by simple organic compounds. Can. J. Microbiol. 22:719-730.

43. Tuttle, J.H., P.R. Dugan, and W.A. Apel. 1977. Leakage of cellular material from **Thiobacillus ferrooxidans** in presence of organic acids. Appl. Environ. Microbiol. 33:459-469.

44. Vogler, K.G. and W.W. Umbreit. 1941. The necessity for direct contact in sulfur oxidation by **Thiobacillus thiooxidans**. Soil Science 51:331-337.